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

MANAL SAMI EL SAYED HASSAN

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

submitted by **MANAL SAMI EL SAYED HASSAN** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Biochemistry, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _______________

Assoc. Prof. Dr. Yeşim Soyer
Head of the Department, **Biochemistry** _______________

Prof. Dr. G. Candan Gürakan Gültekin
Supervisor, **Food Engineering, METU** _______________

Prof. Dr. Ayşe Gül Gözen
Co-Supervisor, **Biology, METU** _______________

Examining Committee Members:

Prof. Dr. Alev Bayındırlı
Food Engineering, METU _______________

Prof. Dr. G.Candan Gurakan Gultekin
Food Engineering, METU _______________

Prof. Dr Kamuran Ayhan
Food Engineering, Ankara University _______________

Assoc. Prof. Dr. Yeşim Soyer
Food Engineering, METU _______________

Assist. Prof. Dr. Aysun Cebeci
Materials Science and Nanotechnology Engineering,
Abdullah Gül University _______________

Date:24.09.2020

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: MANAL SAMI EL SAYED HASSAN

Signature :

ABSTRACT

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

Hassan, Manal Sami El Sayed

Doctor of Philosophy, Biochemistry
Supervisor: Prof. Dr. G. Candan Gürakan Gültekin
Co-Supervisor: Prof. Dr. Ayşe Gül Gözen

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 MIKROORGANIZMALARIN RT-PCR İLE TESPITI VE *LACTOBACİLUS* TÜRLERININ IZOLASYONU TANISI, PROBIOTIC ÖZELLİKLERİ

Hassan, Manal Sami El Sayed

Doktora, Biyokimya
Tez Yöneticisi: Prof. Dr. G. Candan Gürakan Gültekin
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 tespite 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. delbrueckiisubsp. bulgaricus, L. fermentum, L. plantarum, L. amylovorus, Streptococcus thermophilus* ve *Saccharomyces cerevisiae*'ın birkaç kefir örneğinde tespit edildiğini göstermektedir. Mikroskobik 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 birlikle, *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 alignement), bazı izolatların tanısında yararlı olmuştur.

Anahtar Kelimeler: Kefir, *Lactobacillus,* gerçek zamanlı PZR, *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,* 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

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

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 realted to the existence of metabolic substancesas 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’Connel (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 organolepyic 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 eleminating 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 develope 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*, *Staphylococcu*, *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 fumicutes. The genera *Lactococcus, Lactosphera, 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 *Lactobabillus* (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.

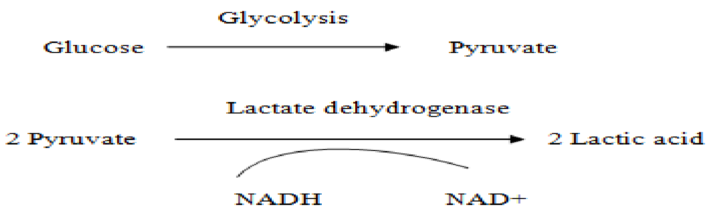
1.2.2 Description of Lactic Acid Bacteria

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

$$\text{Acetaldehyde} + \text{NADH} + H^{+} \longrightarrow \text{Ethanol} + \text{NAD}^{+}$$

$$\text{Glucose} \longrightarrow \text{Lactic acid} + \text{Ethanol} + \text{Carbon dioxide}$$

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 microrganisms 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 substrateas 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 week 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 properities 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 detemine 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 intermediry 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 H_2O_2. 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^oC to 45^o 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 (Tm).

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

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

L. acidophilus *L. delbreuckii* *L. casei*	Taiwan dairy products	Kao Y. T. et al., 2007
L. acidophilus, L. brevis, *L. delbrueckii* subsp. *bulgaricus,* *L. helveticus, L. reuteri*	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 (Querci et al., 2010).

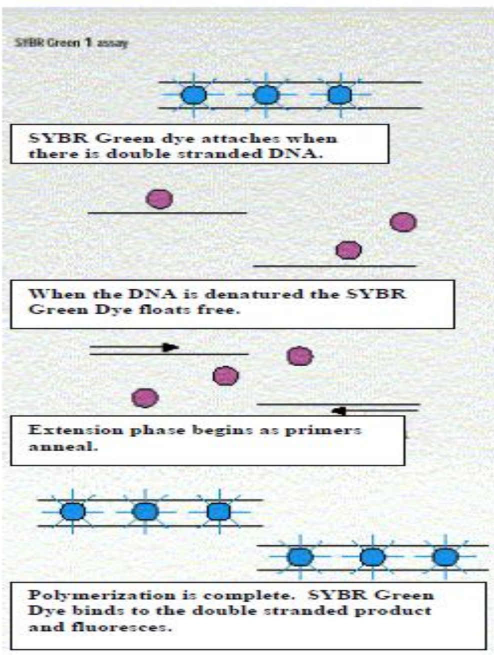


Figure 1.2 SYBR Green I real-time PCR assay (applied biosystems)

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'Connel (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 kefyr* and *S. cerevisiae* are the predominant microrganisms (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'Connel, 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 mechanismis 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. *pseudoplantarum* 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. *pseudoplantarum* 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).

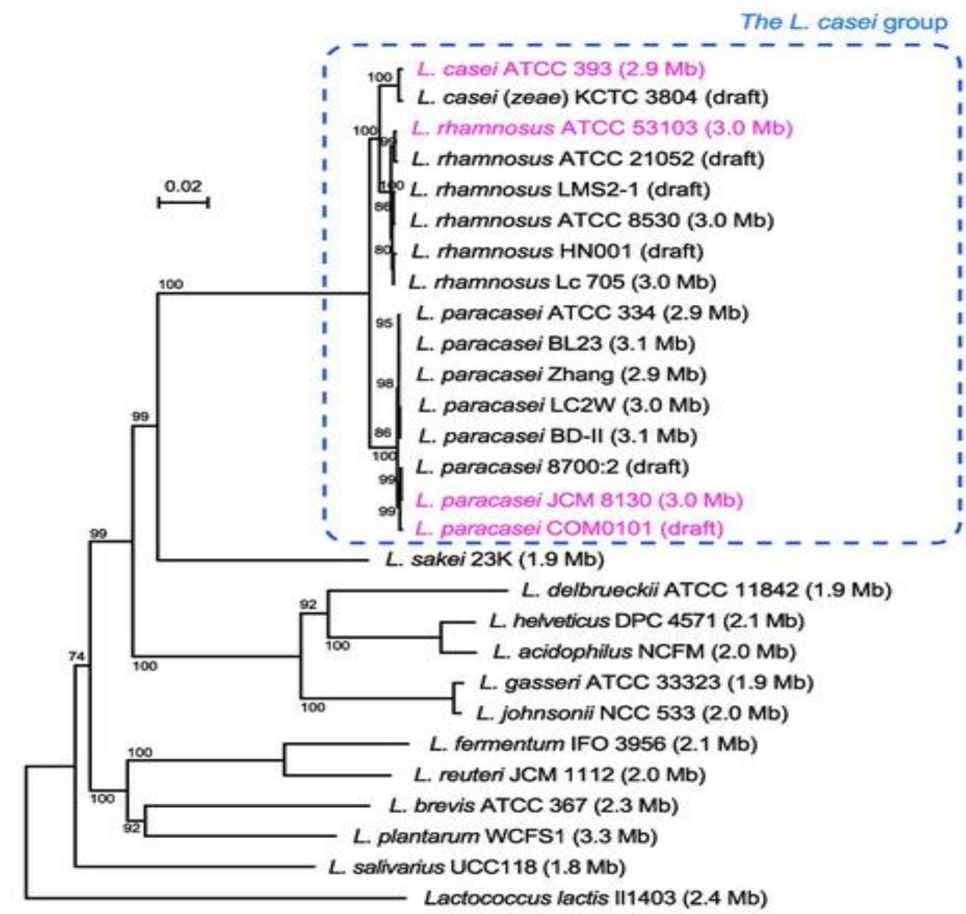


Figure 1.3 Genome-based phylogenetic analysis of the *L. casei* group (Toh H. et al., 2013).

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	*L. kefiranofaciens, L. plantarum, L. lactis* ssp. *lactis, Kluyveromyces marxianus, S. cerevisiae, L. kefiri, Acetobacter* sp.*, L. parakefiri, L. paracasei*	Garrote et al., 2001; Hamet et al., 2013
Kefir grains and milk	Belgium	*L. kefiri, Lactococcus lactis, L. kefiranofaciens, Lactococcus lactis* ssp. *cremoris, Kluyveromyces marxianus, Kazachastania khefir*	Korsak et al., 2015
Kefir grains and milk	Bulgaria	*L. brevis, L. helveticus, L. casei* ssp. *pseudo plantarum, L. delbrueckii* ssp. *bulgaricus, Str. thermophilus, S. cerevisiae, L. lactis*	Simova et al., 2002
Kefir grains	Brazil	*L. kefiri, Lactococcus lactis, L. paracasei, L. helveticus, L. crispatus, L. kefiranofaciens, S. cerevisiae, Leuconostoc* sp.*, Streptococcus* sp.*, Acetobacter* sp.*, Bifidobacterium* sp.*, L. amylovorus, L. parakefiri*	Leite et al., 2012; Zanirati et al., 2015
Kefir milk	Brazil	*L. paracasei, L.casei, L. kefiri, Lactococcus lactis, Kluyveromyces lactis, L. parabuchneri kazachstania aerobia, S. cerevisiae, Lachancea meyersii*	Magalhaes et al., 2011

Kefir grains	Taiwan	*L. kefiranofaciens, L. kefiri, Leuconostoc mesenteroides, Saccharomyces turicensis, Lactococcus lactis*	Chen et al., 2008; Wang et al., 2012

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

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

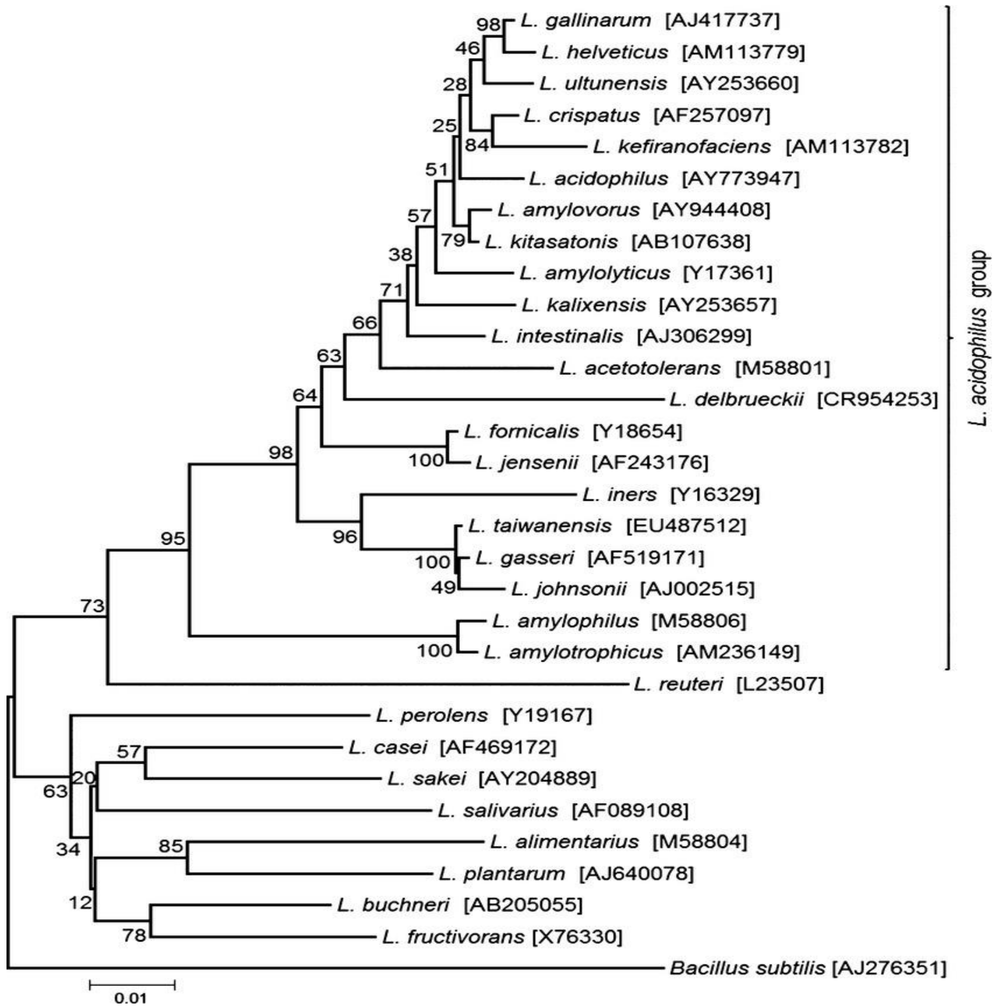


Figure 1.4 Phylogenetic placement of *L. acidophilus* subgroup with in the *Lactobacillus* genus (Matthew B, 2013).

1.5.10 *Lactobacillus kefiri*

L. kefiri is a *Lactobacillus* strain, it has some antibiotic resistence, 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. kefiri*.

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 Properities 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 substanecs 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	Beypazari
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 transfered to pasteurized milk. The milk was covered, placed in warm conditions (30^{o}C). After that, the milk was incubated until lightly thickened and aroma was pleasant. It takes 24 hours in room temperatures (25-30^{o}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^{o}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. kefiri, L. casei, L. delbrueckii* subsp. *bulgaricus, L. kefiranofaciens, L. fermentum, L. plantarum, S. cerevisiae, S. thermophilus, L.sobris \amylovorus, L. acidophilus* and *L. paracasei* (Table 2.2).

Table 2.2 The oligonucleotides primers.

	Strain	Sequence	References
1	*L. acidophilus*	For: CTTTGACTCAGGCAATTGCTCGTGAAGGTAG Rev: CAACTTCTTTAGATGCTGAAGAAACAGCAG CTACG	Herbel (2013)
2	*L. kefiri*	For: 5'GGGAGATGCCCATGTTGGT-3 Rev: 5'AAGCTTTCGAAGTGCCTGTGA-3	Kim et al., (2016)
3	*L. delbrueckii* subsp. *bulgaricus*	For: GAACTTGATGTTGTTGAAGGGATGCAATTCG Rev: GAGCGGCCTTGTTGCACGATTTC(439-461)	Herbel (2013)
4	*L. kefiranofaciens*	For: 5CAGTTCGCATGAACAGCTTTTAA-3 Rev: 5'-GCACCGCGGGTCCAT-3'	Dong et al., (2015)
5	*L. fermentum*	For: AAC CGA GAA CAC CGC GTT AT Rev: ACT TAA CCT TAC TGA TCG TAC ATC AGT CACA	Monique and Jan (2006)
6	*L. plantarum*	For: TGG ATC ACC TCC TTT CTA AGG AAT Rev: TGT TCT CGG TTT CAT TAT GAA AAA ATA	Monique and Jan (2006)
7	*L. sobrius\ amylovorus*	For: TTCTGCCTTTTTGGGATCAA Rev: CCTTGTTTATTCAAGTGGGTGA	Romain Marti et al., (2010)
8	*Streptococcus thermophilus*	For: GGTCCAAGAAGAAGTAATTGA Rev: GACCTTATACAAATCTGGTT	Abd El -Aziz et al., (2014)
9	*Saccharomyces cerevisiae*	For: ACATATGAAGTATGTTTCTATATAACGGGTG Rev: TGGTGCTGGTGCGGATCTA	Abd El-Aziz et al., (2014)
10	*L. casei*	For: CTA TAA GTA AGC TTT GAT CCG GAG ATT Rev: CTT CCT GCG GGT ACT GAG ATG T	Monique and Jan (2006)
11	*L. paracasei*	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_2O	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 specifity of real time PCR reaction.

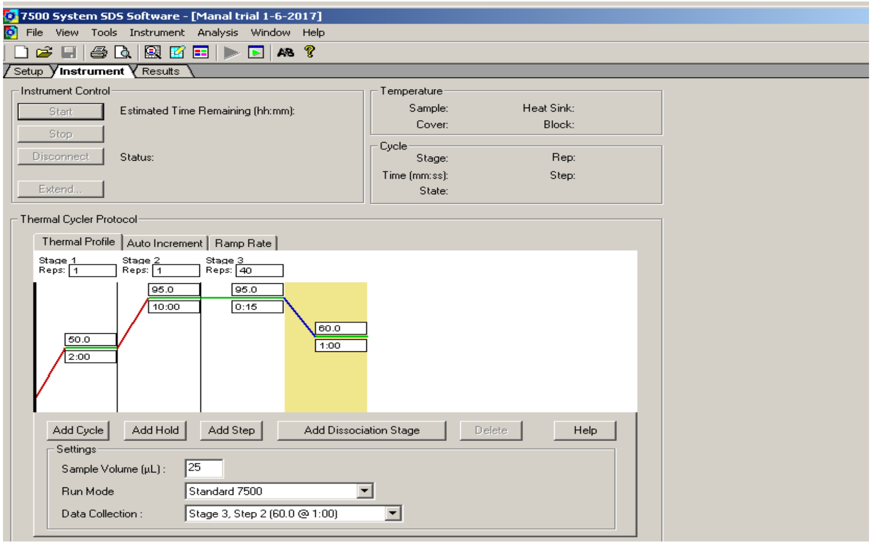


Figure 2.1 Real-time PCR program

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 preseved 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. Afterthat 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 transfered 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-μL 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_2O	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.

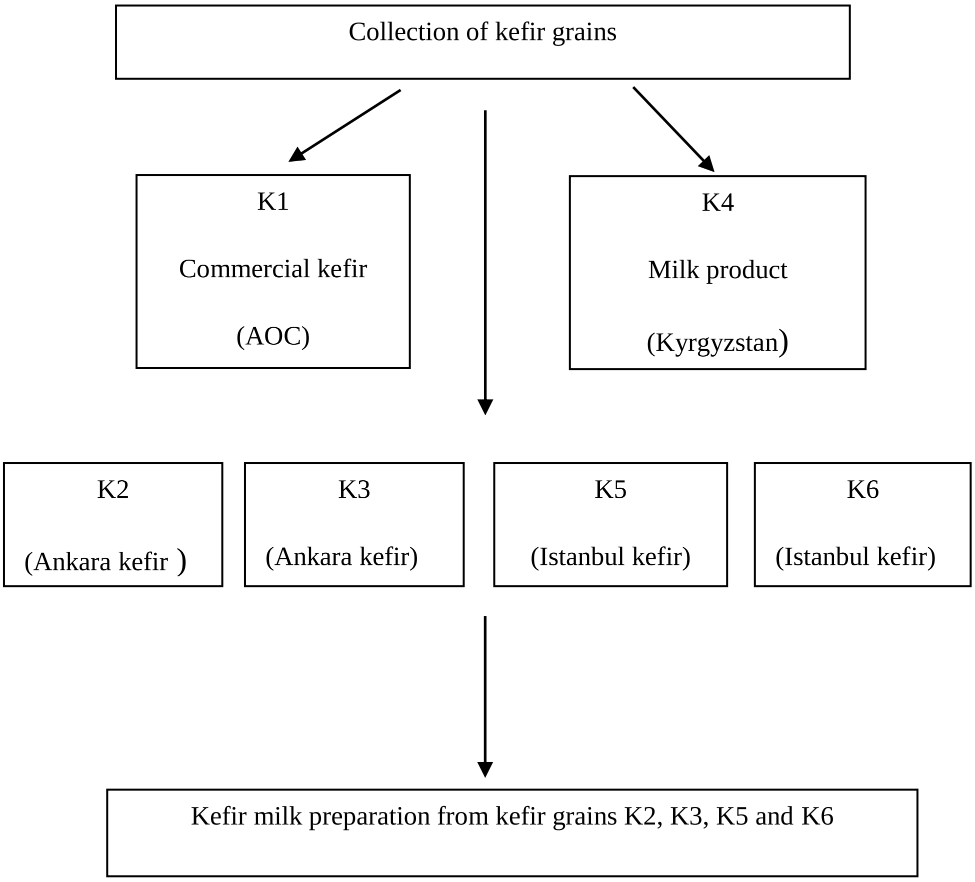


Figure 3.1 Experimental planing (Section 1); collections of kefir grains and preparation kefir milk.

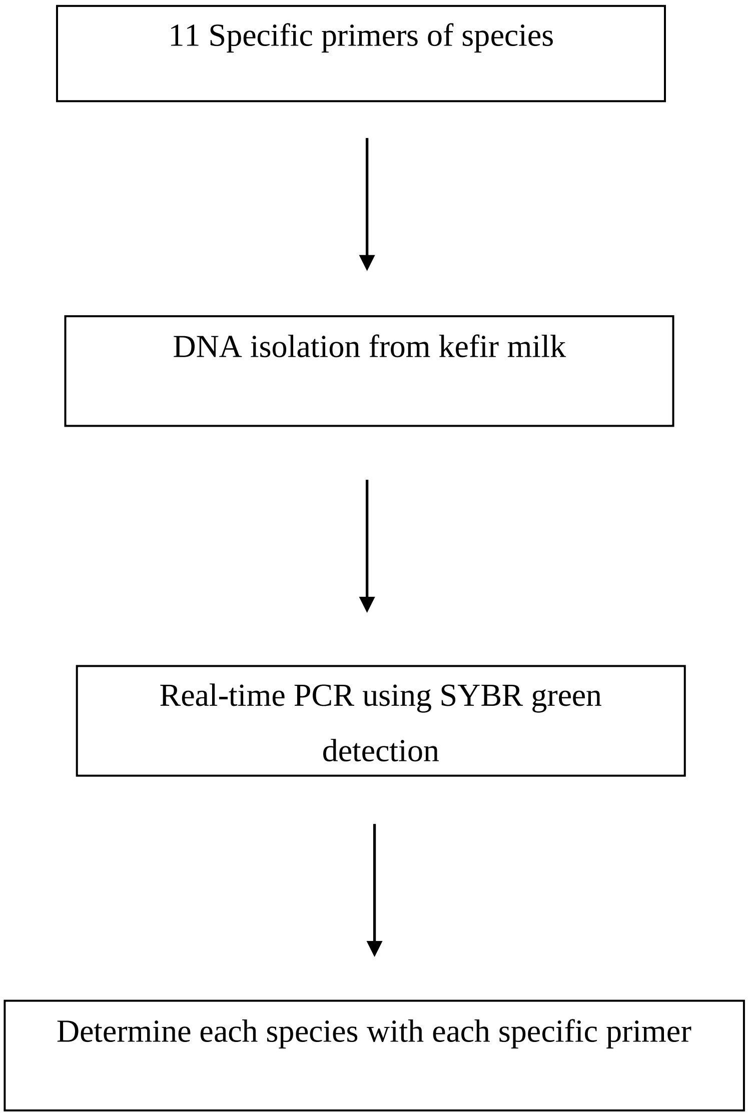


Figure 3.2 Experimental planning (Section 2); determination of the population in homemade kefir samples

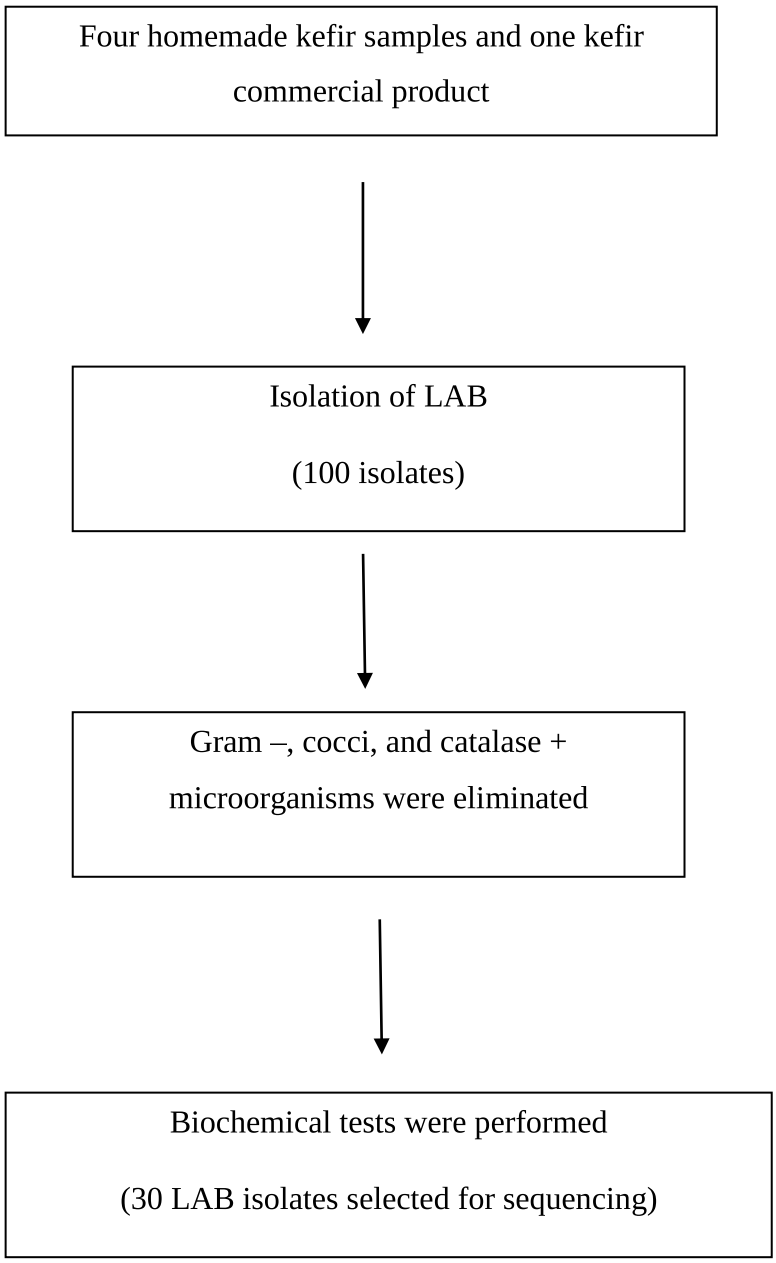


Figure 3.3 Experimental planning (Section 3); isolation and microbiological / biochemical identifications of LAB.

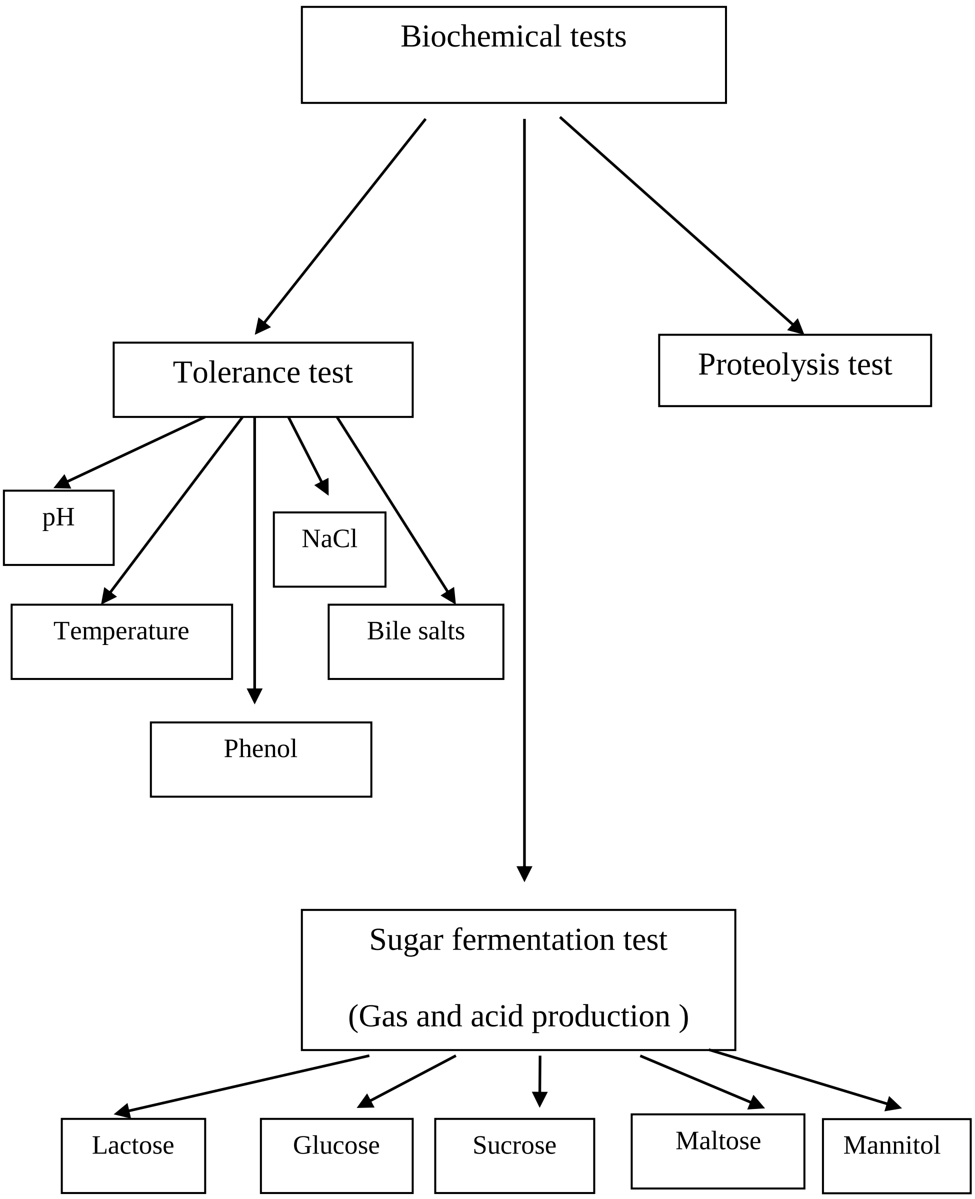


Figure 3.4 Biochemical tests performed in the study (Section 4)

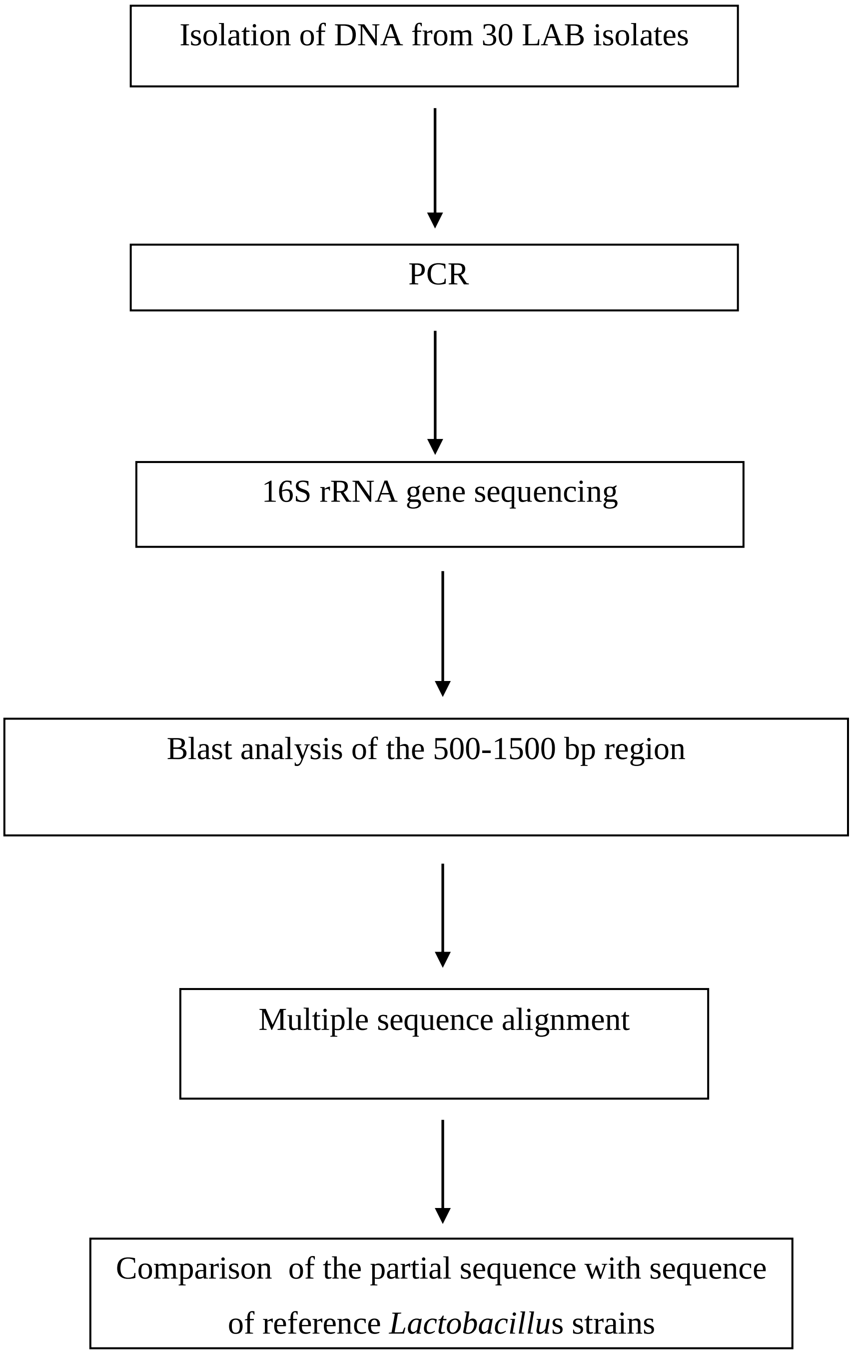


Figure 3.5 Experimental planning of the blast analysis

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^{o}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^{o}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. kefiri, L. delbrueckii* subsp. *bulgaricus, L. acidophilus, L. kefiranofaciens, L. fermentum, L. plantarum, S. cerevisiae, S. thermophilus, L. sobris \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. kefiri, 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 *L. kefiri***	**P2 *L. acidophilus***	**P3 *L. delbrueckii* subsp *bulgaricus***	**P4 *L. kefiranofaciens***	**P5 *L. fermentum***	**P6 *L. plantarum***	**P7 *S. cerevisiae***	**P8 *S. thermophilus***	**P9 *L. sobris \amylovorus***	**P 10 *L. casei***	**P 11 *L. paracasei***
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 (ΔRn) against the cycle number. The threshold cycle (Ct) 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 (Tm) in which the amplified PCR products were denatured and cooled to 5-10°C. The Tm 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 Tm for *Lactobacillus* spp that was identified from kefir and milk samples using real-time PCR was between 78-85.5^{o}C. The present results are similar to the results by Abdulamir et al (2010) in which the melting curve for *Lactobacillus* spp. in six samples of fish and shrimp sauces had a peak at Tm = 85.5°C.

Lactobacillus kefiri

In the current study, *L. kefiri* 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. kefiri* 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).

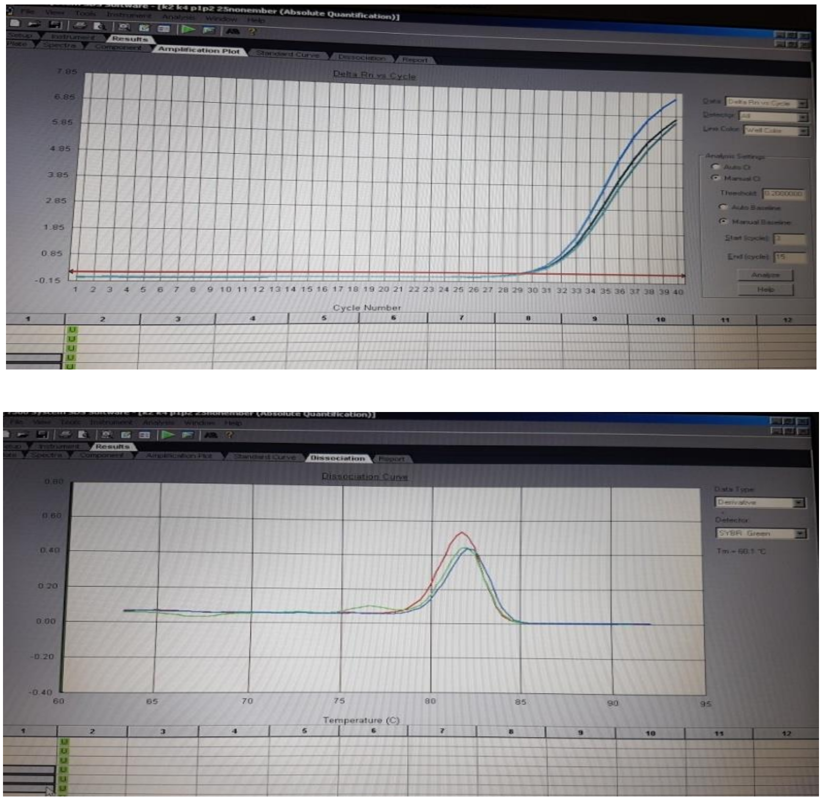


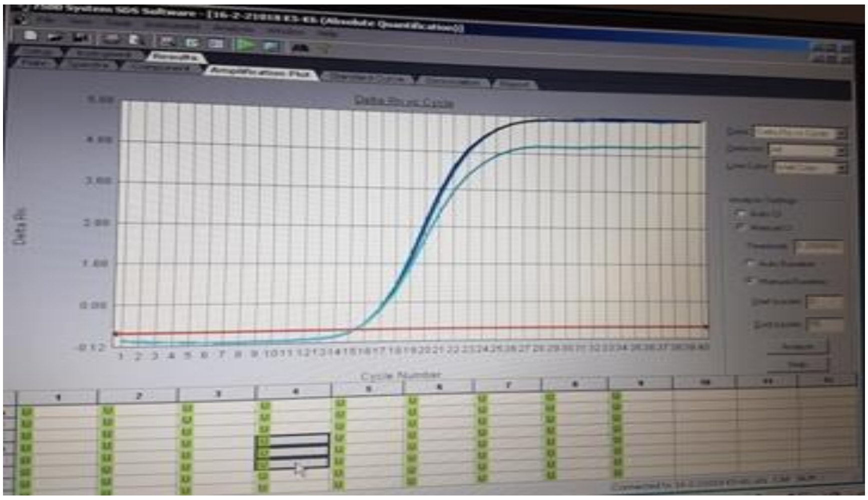
Figure 3.6 Amplification and melting curve for Kefir sample (K4) with *L. kefiri*.

L. kefiri was specified as a starter culture and determines one of the flavor properties of kefir beverages. The presence of *L. kefiri* in all samples in a recent study was demonstrated with real-time PCR analysis. Also, *L. kefiri* 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. kefiri* in the K4 sample had Ct value started from 32 and a specific peak at Tm =82°C.

Lactobacillus kefiranofaciens

Chon et al (2013) studied that the level of *L. kefiranofaciens* was strongly correlated with the amount of kefiran 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)



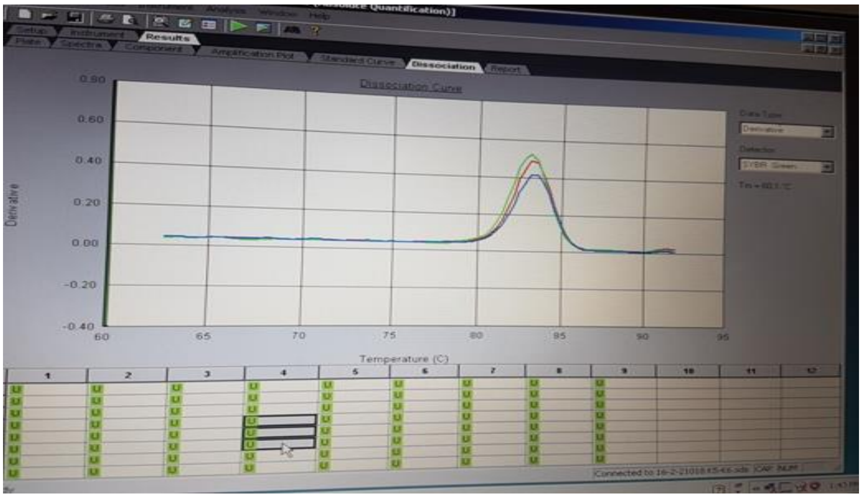


Figure 3.7 Amplification and melting curve for Kefir sample (K6) with *L. kefiranofaciens*.

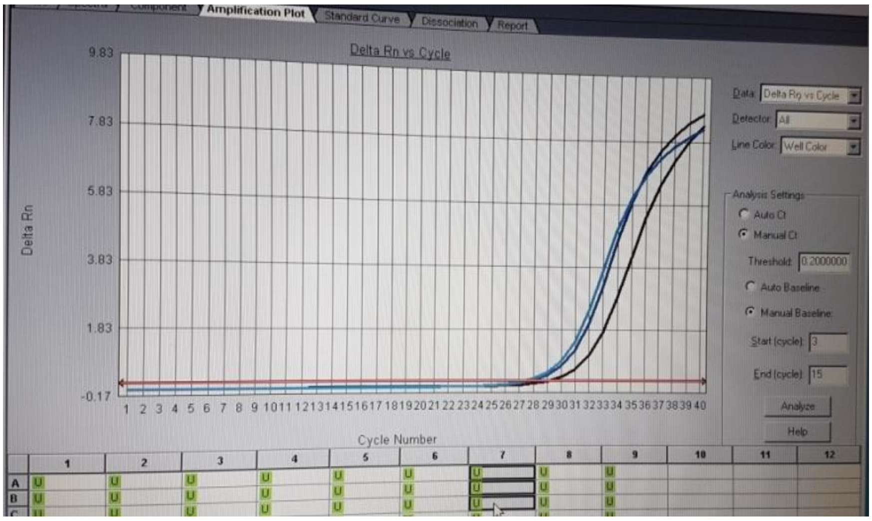
Saccharomyces cerevisiae

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 CO_2 gas in the presence of oxygen, or it can do fermentation in anaerobic conditions to produce ethanol and CO_2 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 kefiran, 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.



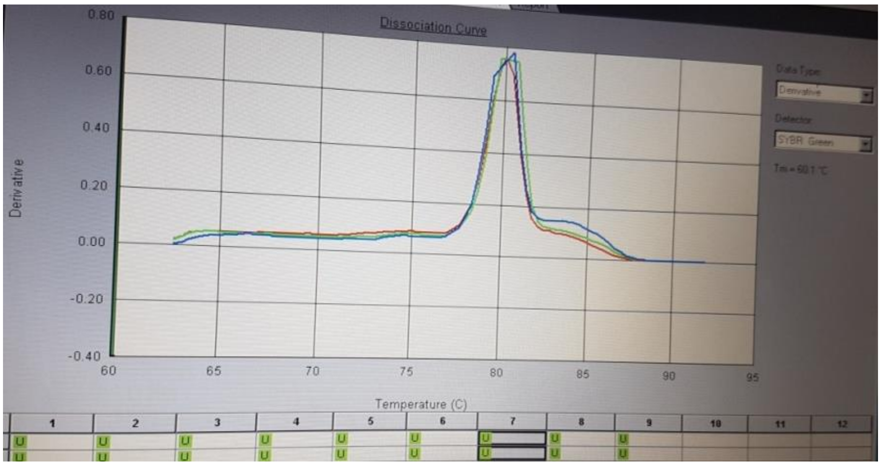


Figure 3.8 Amplification and melting curve for Kefir sample (K5) with *S. cerveisiae.*

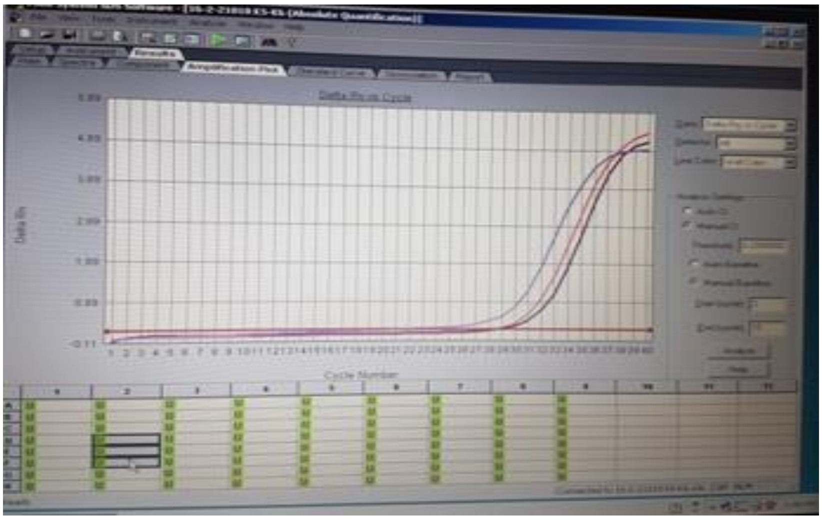
Lactobacillus acidophilus

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 Tm as shown in Figure 3.9 (a). It was considered as a negative result because of the presence of nonspecific products.

The melting curves for *L. acidophilus* had a Ct value at 30 and a specific peak for the melting curve at 83°C. Figure 3.9 (b) shows amplification and melting curves for *L. acidophilus* in K2.



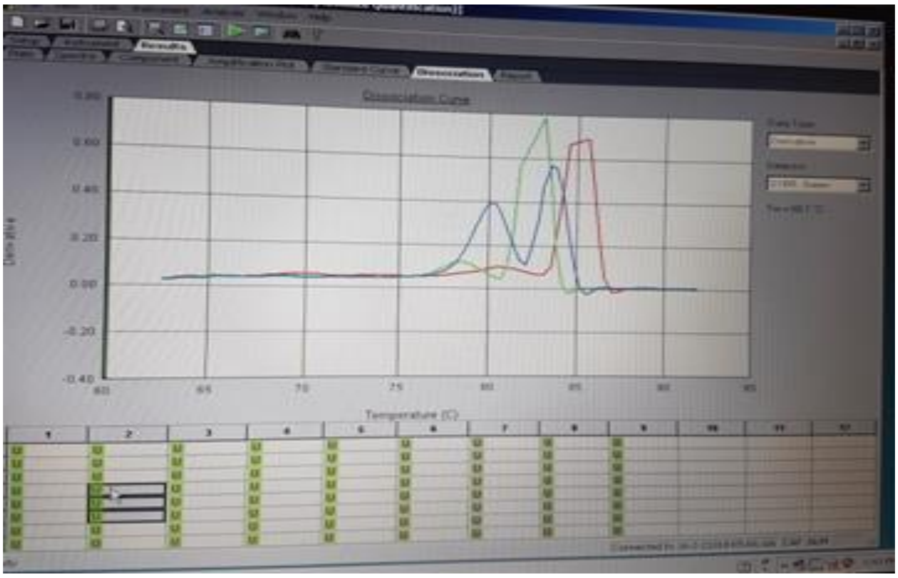
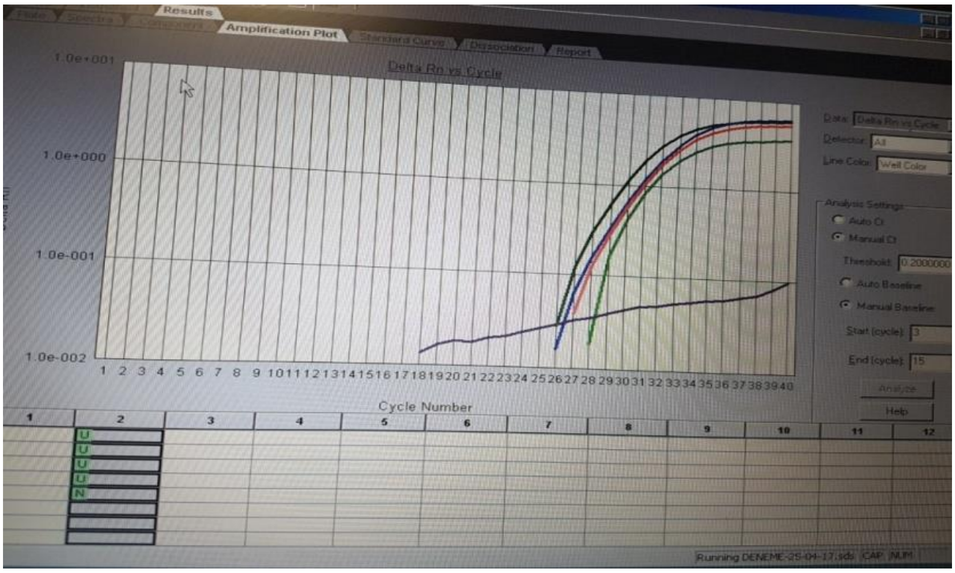


Figure 3.9 (a) Amplification and melting curve for *L. acidophilus* in Kefir (K6), case of primer dimer.



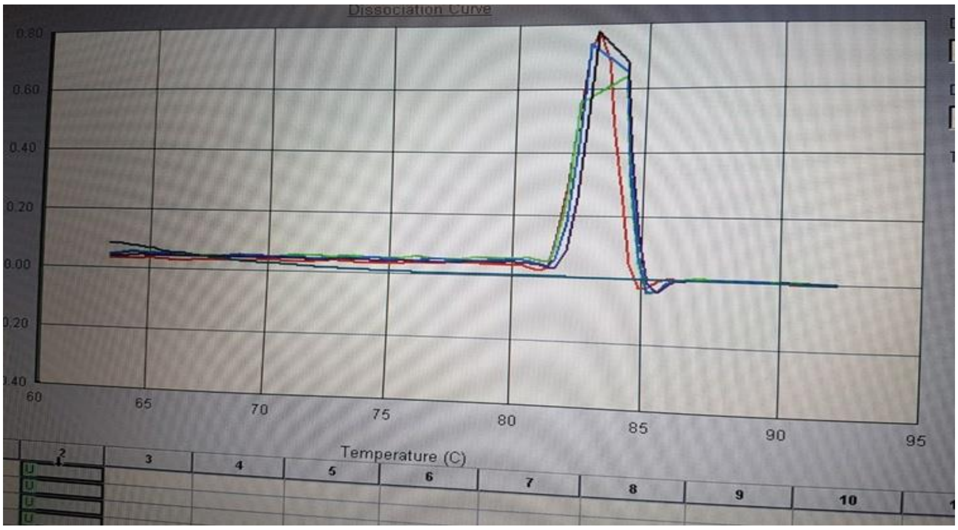


Figure 3.9 (b) Amplification and melting curves for *L. acidophilus* in K2.

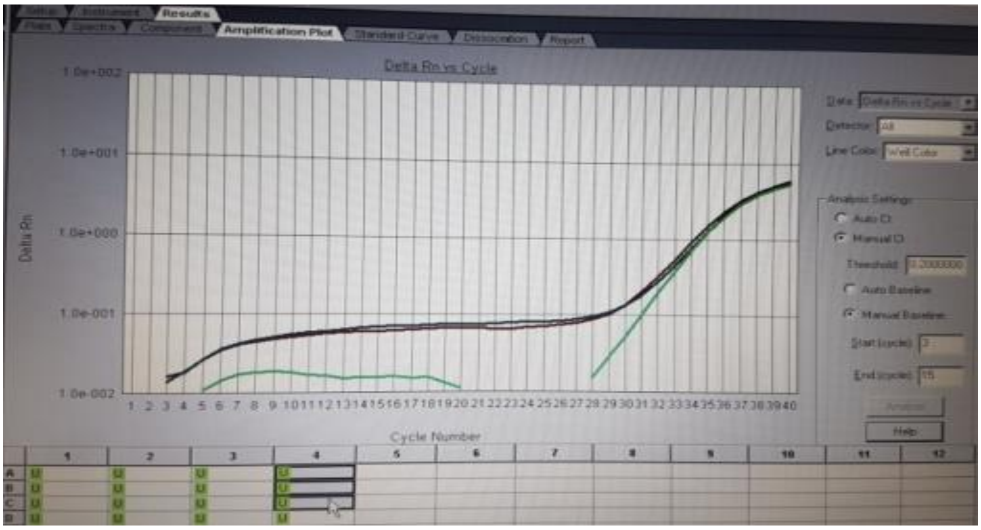
Lactobacillus plantarum

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 (Garrrote 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^{O}C. The amplification and melting curve for *L. plantarum* in K1 and K2 can be seen in Figure 3.10(a,b).



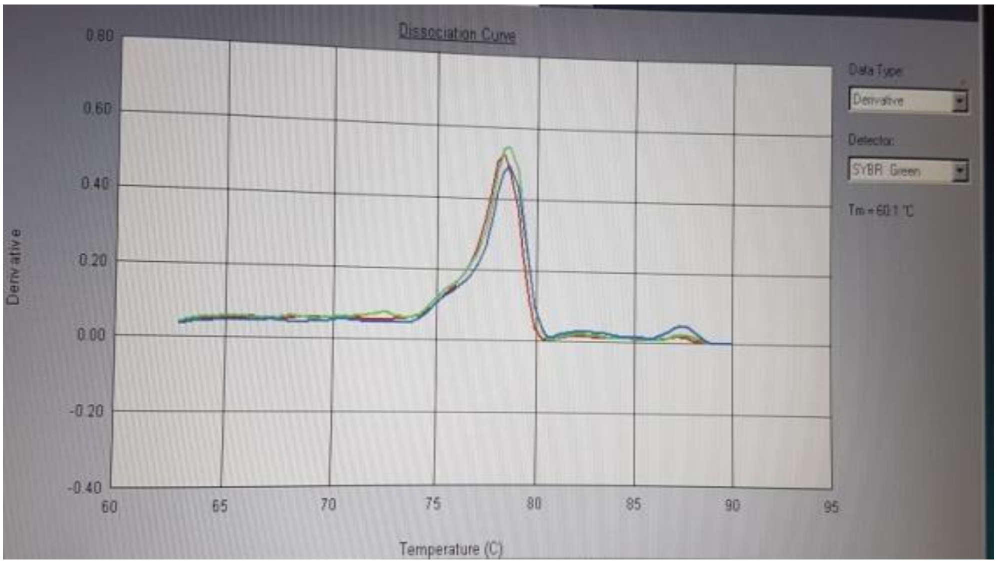
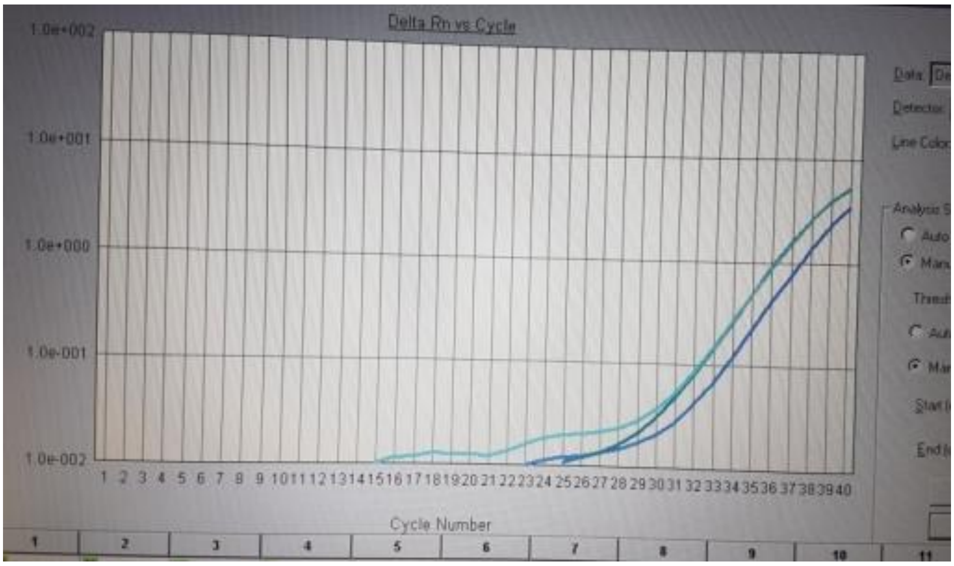


Figure 3.10 (a) Amplification and melting curve for Kefir (K1) with *L. plantarum.*



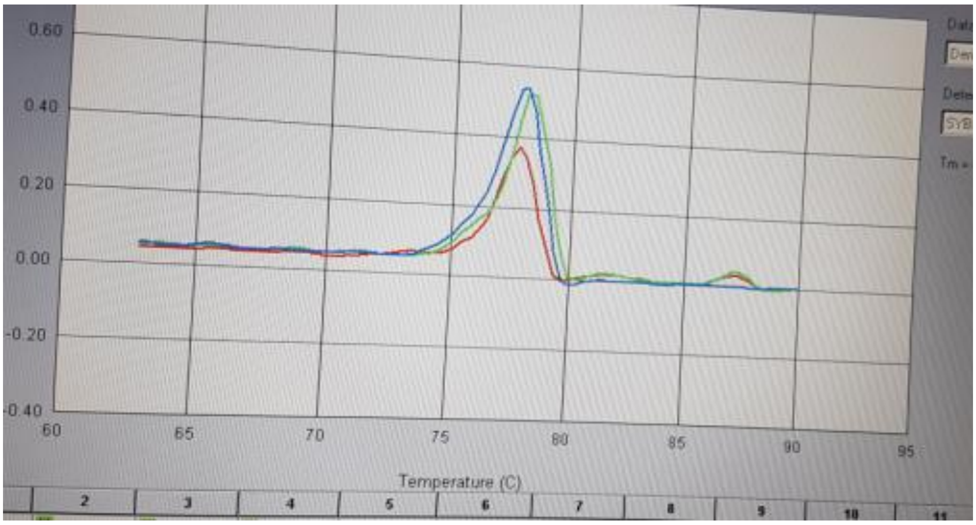


Figure 3.10 (b) Amplification and melting curve for Kefir (K2) with *L. plantarum.*

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 Tm value was located between 82^{o}-85^{o}C. Kao et al (2007) found that Tm 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.

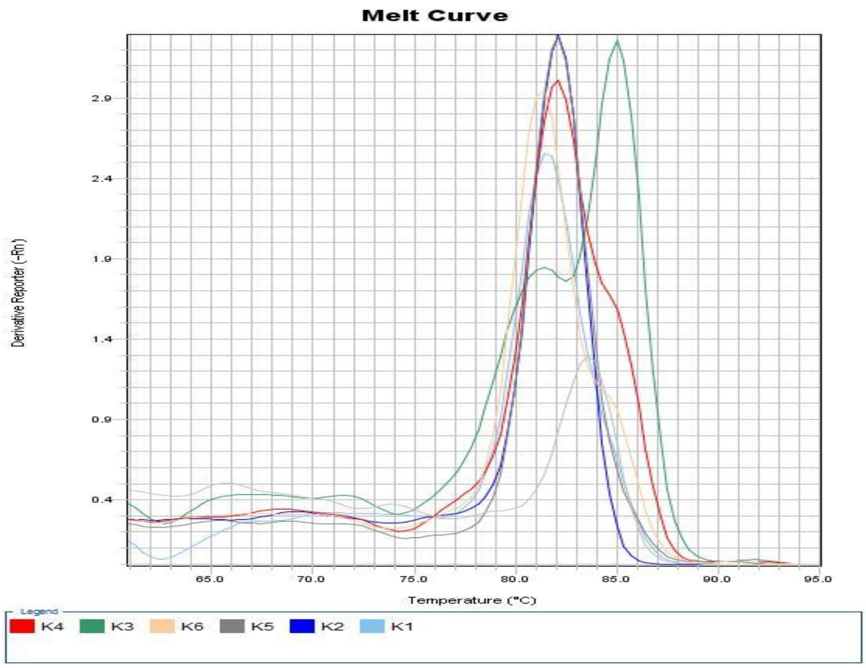
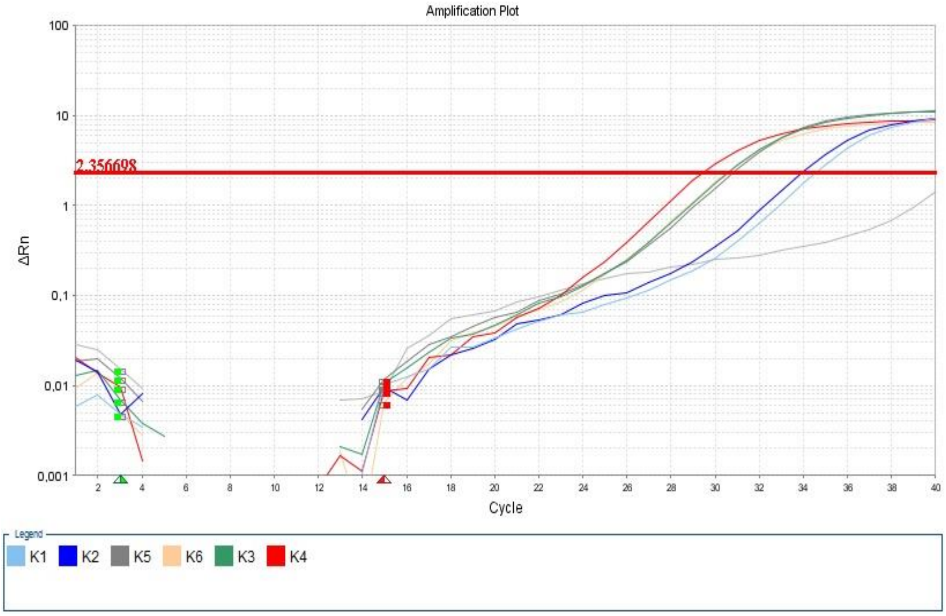


Figure 3.11 Amplification and melting curve for *L. casei* in all samples.

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^{O}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.



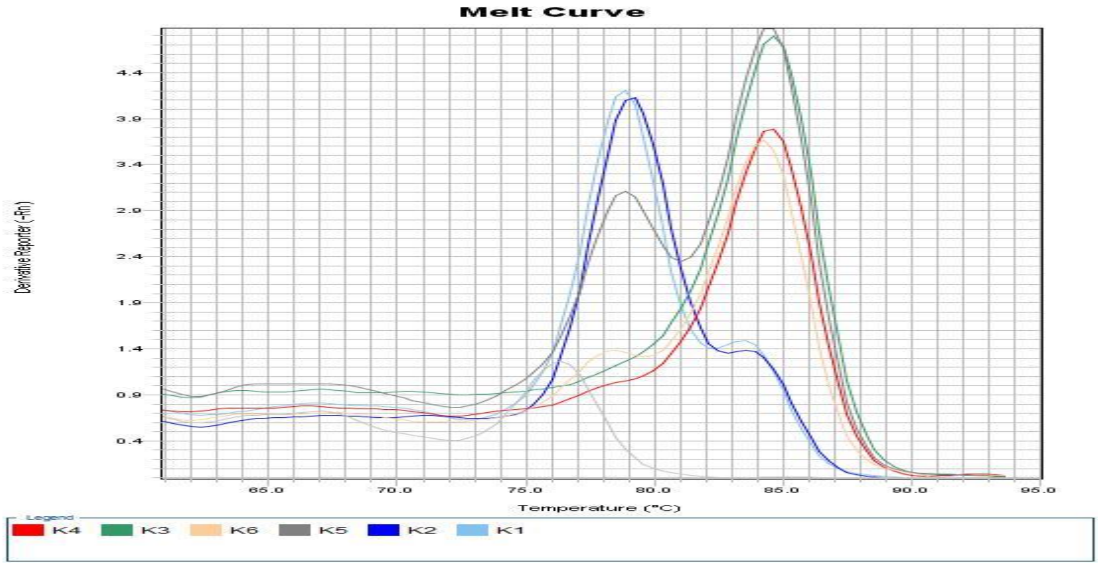


Figure 3.12 Amplification and melting curve for *L. paracasei* in all samples.

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^{O}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^{o}C to 85^{o}C.

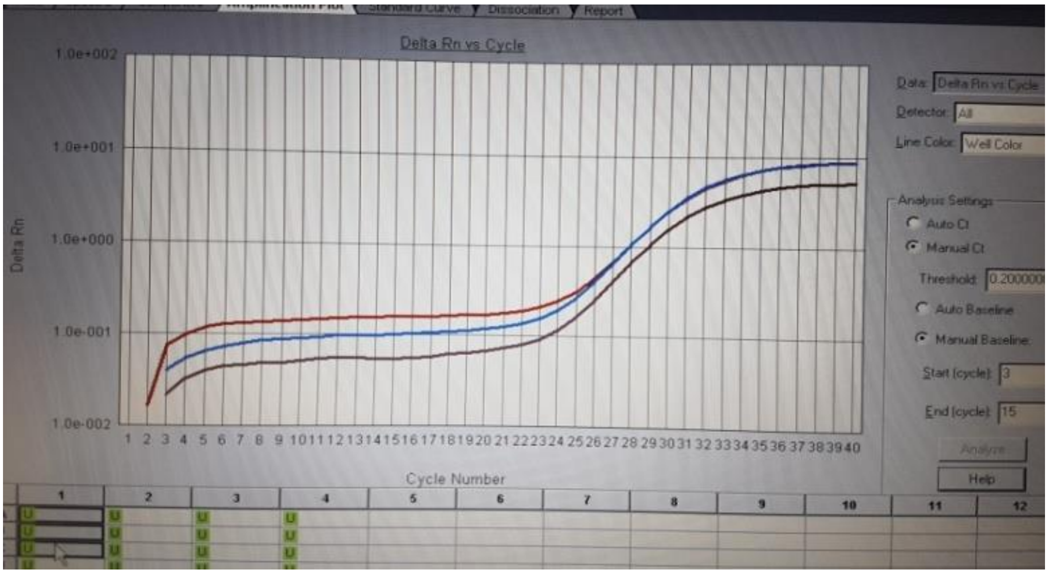


Figure 3.13a The amplification curve of *L. delbrueckii* subsp. *bulgaricus* in k1.

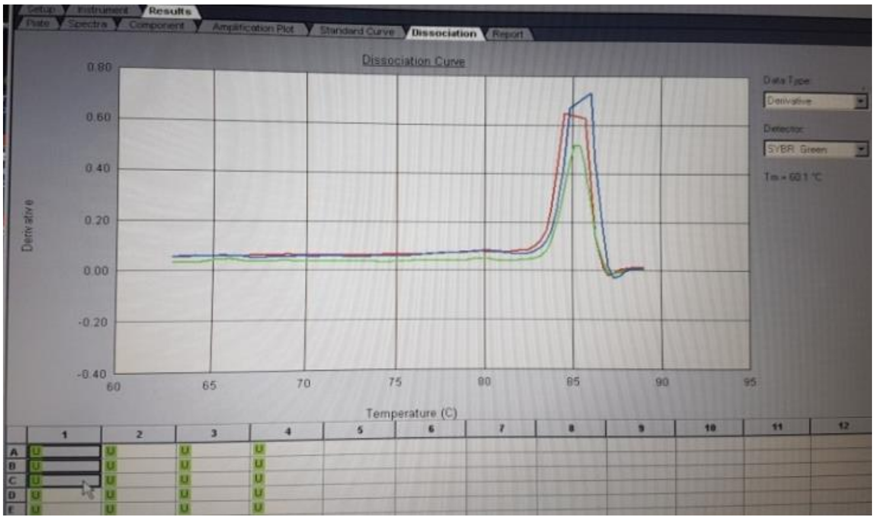


Figure 3.13b The melting curve of *L. delbrueckii* subsp. *bulgaricus* in k1.

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).

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

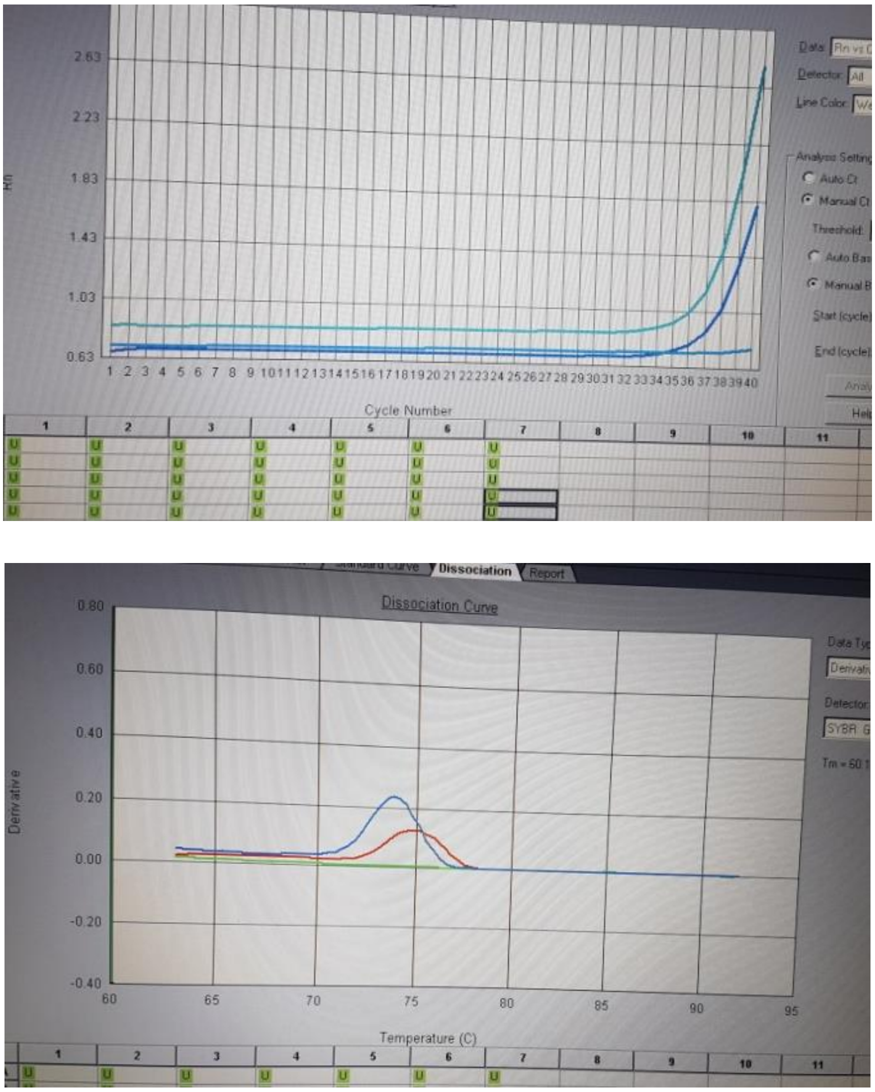


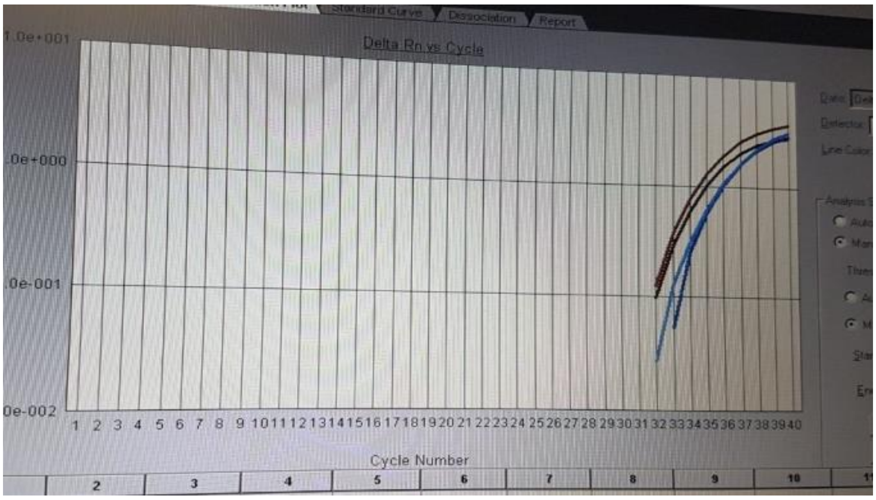
Figure 3.14 The amplification and melting curve of *L. amylovorus* in K4.

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^{o}C in the melting temperature. The amplification and melting curve for K4 can be seen in Figure 3.15. *L. fermentum* is obligately hetero-fermentative 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 kefirs 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.



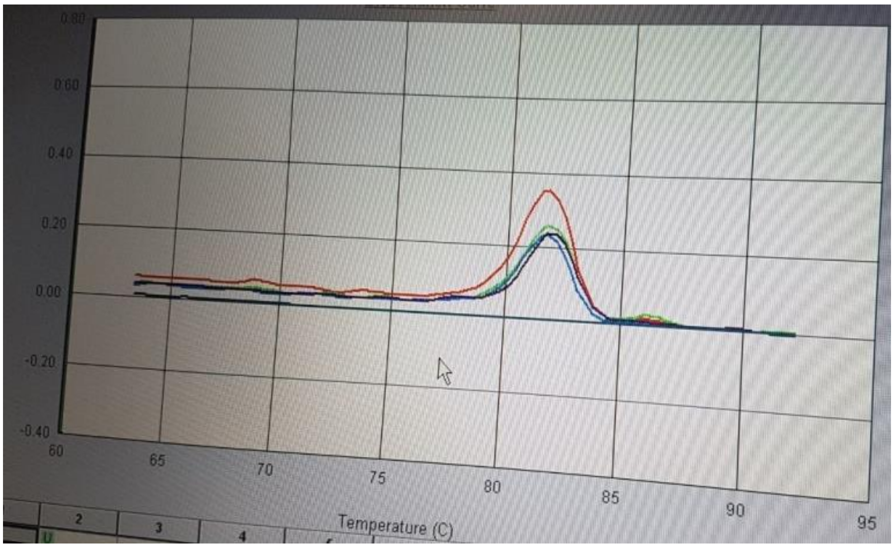


Figure 3.15 The amplification and melting curve of *L. fermentum* in K4.

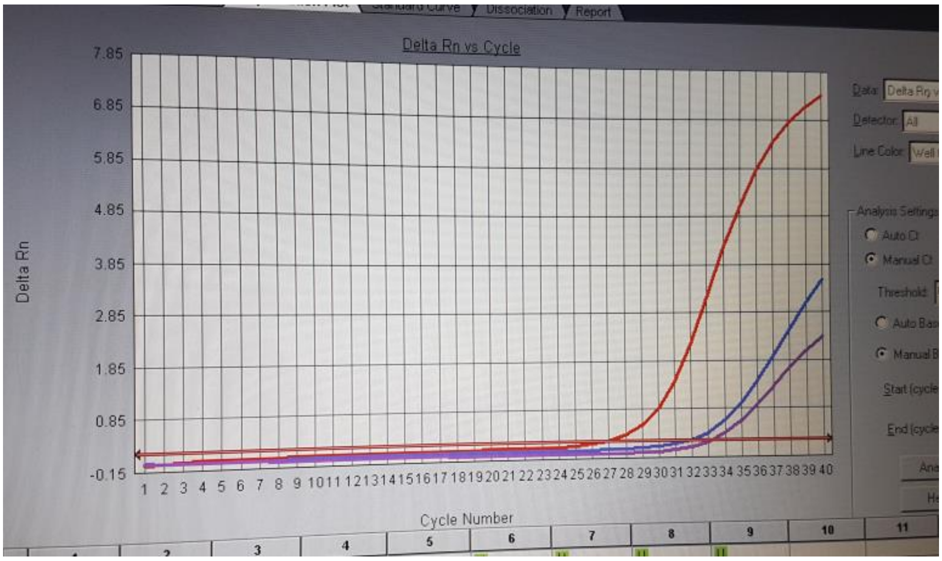
Streptococcus thermophilus

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^{o}C for K1, K5, and K6, but it was 73^{o}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.



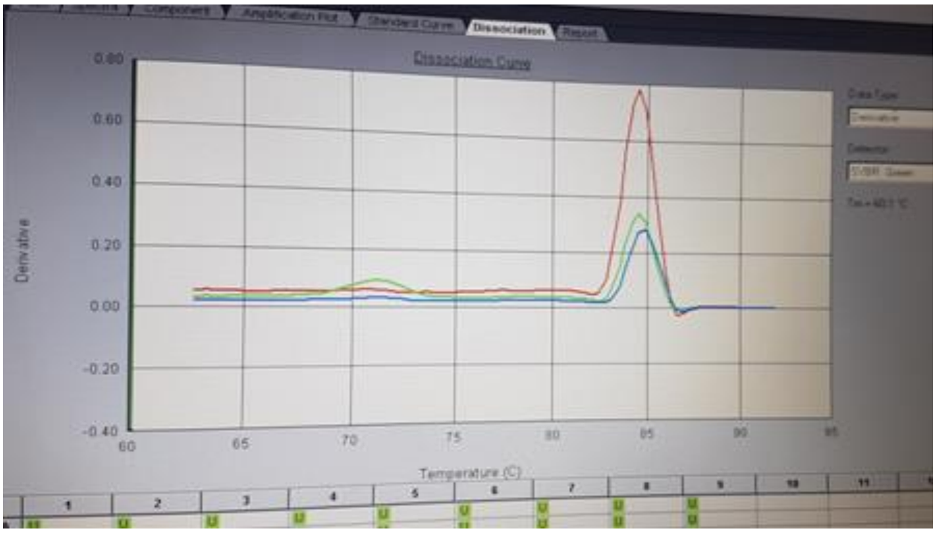


Figure 3.16 The amplification and melting curve of *S. thermophilus* in K6.

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

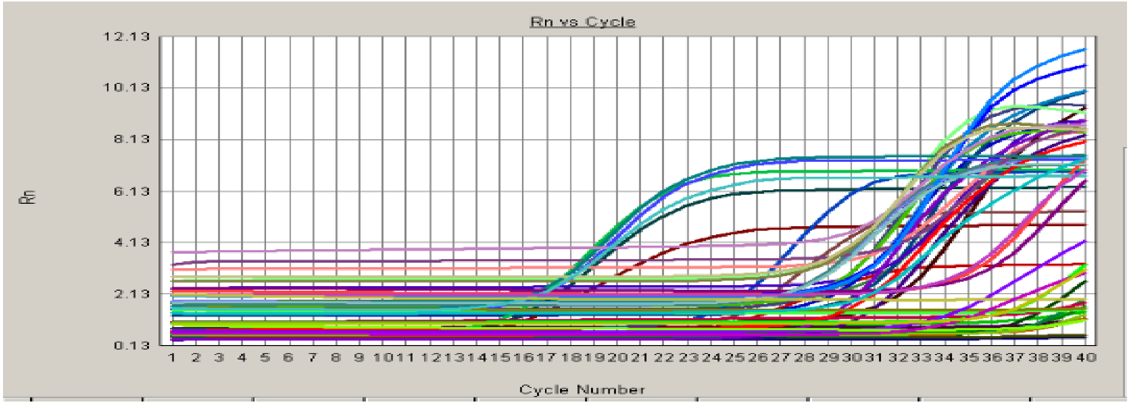


Figure 3.17 (a) Amplification curves of kefir sample (K5 and K6) with specific primers *(L. kefiri, L. acidophilus, L. kefiranofaciens, L. fermentum, L. plantarum, L. delbrueckii* subsp. *bulgaricus, S. cerevisiae, S. thermophilus* and *L. sobris \amylovorus).*

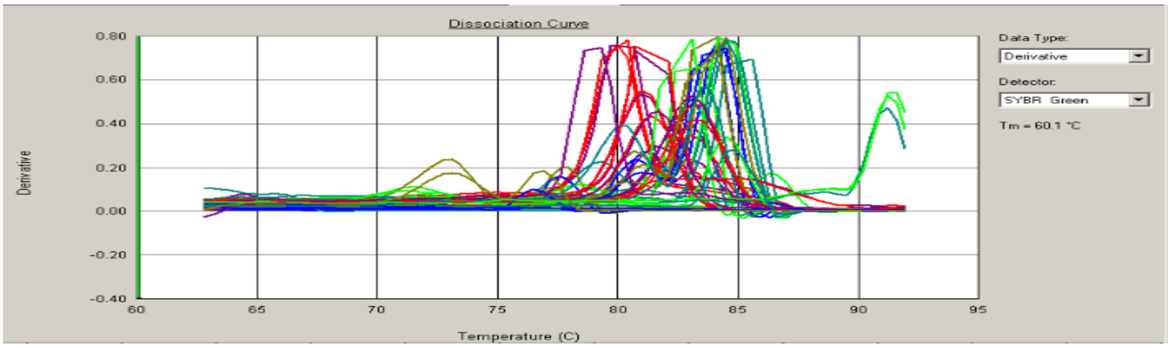


Figure 3.17 (b) Melting curves of kefir sample (K5 and K6) with specific primers (*L. kefiri, L. acidophilus, L. delbrueckii* subsp. *bulgaricus, L. kefiranofaciens, L. fermentum, L. plantarum, S. cerevisiae, S. thermophilus, and L. sobris \amylovorus).*

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. sobris \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

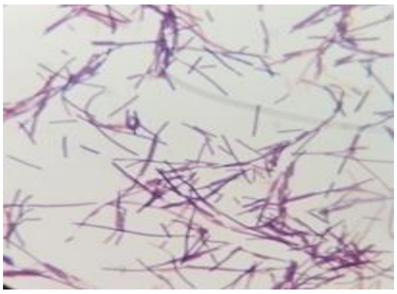
Table 3.2 Sources of kefir and colony morphology of isolates (continued).

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

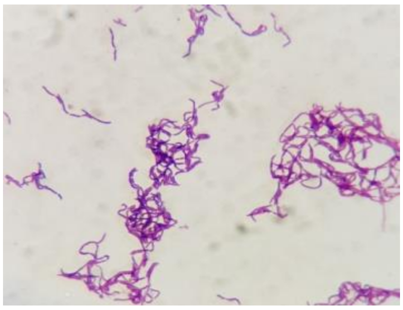
Results are expressed as small for colony diameter 2 mm and large for colony diameter 2-5 mm.

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 ^{o}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 ≤ 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^{o}C (Table 3.5). According to the results, all of the isolates easily grow at 25, 37, and 40^{0}C. These results are similar to those of Pundir et al (2013). However, some isolates could not grow at 45^{o}C. According to Chakraborty and Bhowal (2015), the isolated *Lactobacillus* species were able to survive with in 30-50^{o}C and the optimum temperature for maximum growth was 37^{o}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 25˚C	37˚C	40°C	45˚C	Phenol tolerance (0.4%)	Lactic acid production
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 helps 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^{o}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.

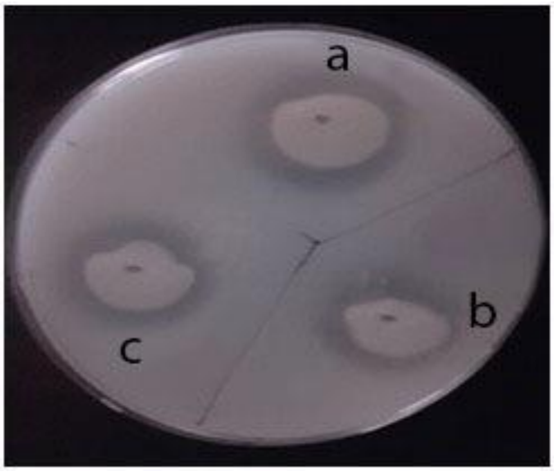


Figure 3.19 Proteolysis activity for *Lactobacillus* isolates.
a (K6-14), b (K2-4a), c (K5-3)

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).

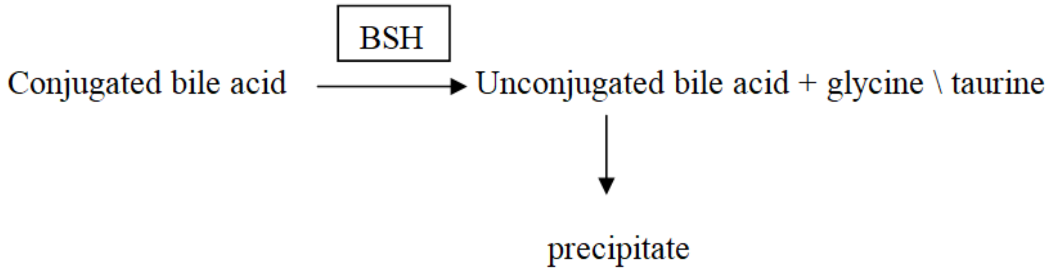
No	Isolate name	Bile salt tolerance test		Proteolysis (diameter of clear zone mm)
		Taurodeoxycholic acid 0.5% (w/v)	Glycocholic acid 0.5% (w/v)	
24	K6-1	+	++	25
25	K6-3a	+	+	30
26	K6-6a	+	+	25
27	K6-7	+	+	29
28	K6-9a	+	+	23
29	K6-12	+	++	21
30	K6-14	++	+	32

(++) 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).

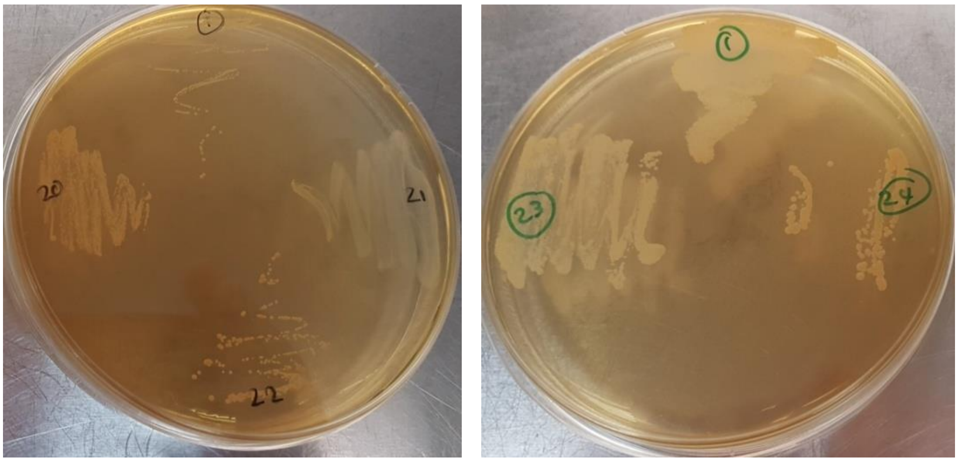


Figure 3.20 Bile salt hydrolase activity by lactobacilli on solid MRS. The greatest precipitations were by 1 (K2-4) , 21 (K4-17), and 23 (k3-13b)

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 durhum 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	+	-	+	+	-	-

Table 3.7 Carbohydrates fermentation patterns of isolates (continued)

Isolate	Isolate	Glucose		Sucrose	Lactose	Maltose	Mannitol
No	name	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 identifiying 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	*L. casei*	Shafakatullah and Chandra 2015
		L. plantarum *L. paracasei*	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	*L.brevis*	Abdel-Rahman et al. 2011
K2-3, k2-4a, k2-14, k3-3a	Glucose (acid and no gas formation), sucrose, lactose, maltose,mannitol	*L. acidophilus*	Hassan et al., 2014
K2-3a, K4-6a, K6-6a	Glucose, sucrose, lactose, maltose	*L. mesenteroides*	Gebreselassie et al., 2016
K3-8b2, k3-28, k6-1	Glucose, sucrose, lactose, mannitol	*L. bulgaricus*	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	*L. brevis*	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.

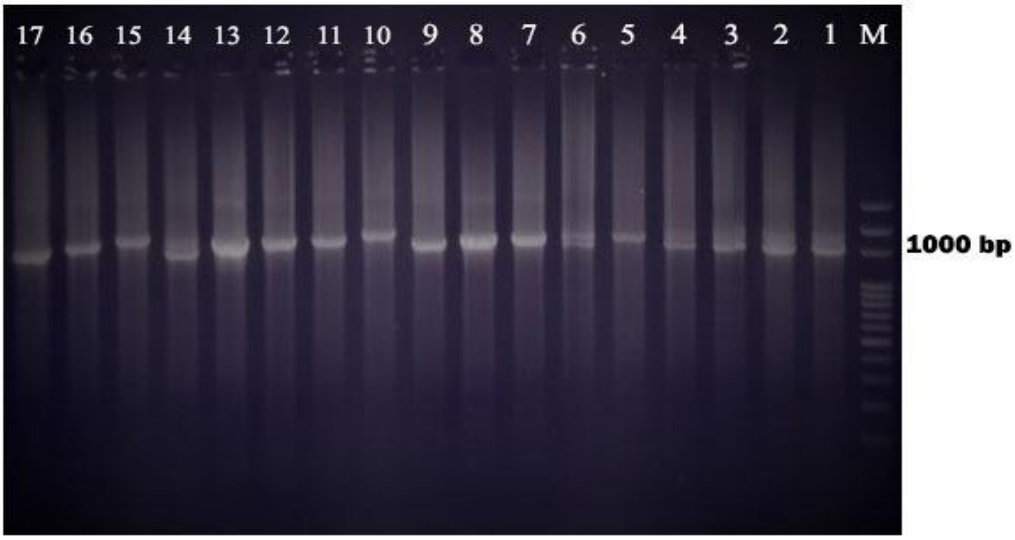


Figure 3.21 Agarose gel electrophoreses of PCR products using 27F - 1492R as universal primers and the marker is the 100 bp DNA. 1(K3-8b2), 2(K6-7), 3(K4-16), 4(K5-3), 5(K3-13b), 6(K4-28), 7(K6-12), 8(K3-20a), 9(K2-4), 10(K3-28), 11(K2-3), 12(K5-11), 13(K3-20a), 14(K4-17), 15(K6-14),16(K5-15),17(K2-14).

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	*L. gallinarum* strain ATCC 33199	NR_042111.1	99.22
		L. helveticus strain NBRC 15019	NR_1137191.1	99.05
		L. acidophilus strain NBRC 13951	NR_113638.1	97.93
2	*K2-3	*L. intestinalis* strain TH4	NR_117071.1	98.27
		L. gallinarum strain ATCC 33199	NR_042111.1	99.19
		L. helveticus strain NBRC 15019	NR_1137191.1	99.01
3	K2-3a	*L. gallinarum* strain ATCC 33199	NR_042111.1	99.45
		L. helveticus strain NBRC 15019	NR_1137191.1	99.36
		L. acidophilus strain NBRC 13951	NR_113618.1	98.08
4	K2-4	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_041054.1	99.67
		L. zeae strain RIA 482	NR_037122.1	98.69
		L. casei 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	*L. gallinarum* strain ATCC 33199	NR_042111.1	99.05
		L. helveticus strain NBRC 15019	NR_113719.1	99.87
		L.crispatus strain DSM 20584	NR_119274.1	98.30
		L. amylovorus DSM 20531	NR_117064.1	98.20
6	K2-14	*L. gallinarum* strain ATCC 33199	NR_042111.1	99.43
		L. helveticus strain NBRC 15019	NR_113719.1	99.24
		L. crispatus 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	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_041054.1	99.73
		L. zeae strain RIA 482	NR_037122.1	98.64
		L. casei strain NBRC 15883	NR_113333.1	98.55
8	K3-3a	*L. gallinarum* strain ATCC 33199	NR_042111.1	99.19
		L. helveticus strain NBRC 15019	NR_113719.1	99.01
		L. acidophilus strain NBRC 13951	NR_113638.1	89.20

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	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_041054.1	99.51
		L. zeae strain RIA 482	NR_037122.1	98.52
		L. casei strain NBRC15883	NR_113333.1	98.44
		L. rhamnosus strain NBRC 3425	NR 113332	98.25
10	K3-13b	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_041054.1	99.23
		L. zeae strain RIA 482	NR_037122.1	98.44
		L. casei strain NBRC 15883	NR_113333.1	98.35
		L. rhamnosus strain NBRC 3425	NR 113332.1	98.34
11	K3-20	*L. paracasei* subsp. *tolerans* strain NBRC 15889	NR_113337.1	99.80
		L. zeae strain RIA 482	NR_037122.1	99.69
		L. casei strain NBRC 15883	NR_113333.1	99.69
		L. rhamnosus strain NBRC 3425	NR 113332	99.69
12	K3-20a	*L.paracasei* subsp. *tolerans* strain NBRC 15906	NR_041054.1	99.75
		L. zeae strain RIA 482	NR_037122.1	98.86
		L. casei strain NBRC 15883	NR_113333.1	98.74
		L. rhamnosus 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	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_041054.1	99.92
		L. zeae strain RIA 482	NR_037122.1	98.91
		L. casei strain NBRC 15883	NR_113333.1	98.82
		L. rhamnosus 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	*L. helveticus* NBRC 15019	NR_113719.1	99.66
		L. gallinarum strain ATCC 33199	NR_042111.1	99.66
		L. acidophilus strain NBRC 13951	NR_113638.1	98.37
15	K4-16	*L. paracasei* strain ATCC 25302	NR_117987	99.73
		L. zeae strain RIA 482	NR_037122.1	98.98
		L. casei strain NBRC 15883	NR_113333.1	98.65
16	K4-17	*L. paracasei* subsp. *tolerans* strain NBRC 15889	NR_113337.1	99.81
		L. zeae strain RIA 482	NR_037122.1	99.72
		L. casei strain NBRC 15883	NR_113333.1	99.72
17	K4-28	*L. paracasei* strain NBRC 15906	NR_041054.1	99.50
		L. zeae strain RIA 482	NR_037122.1	98.49
		L. casei 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	*L. paracasei* strain NBRC 15906	NR_041054.1	99.43
		L. zeae strain RIA 482	NR_037122.1	98.45
		L. casei strain NBRC 15883	NR_113333.1	98.37
		L. rhamnosus strain NBRC 3425	NR_113332.1	98.05
19	K5-3a	*L. paracasei* strain NBRC 15889	NR_113337.1	99.75
		L. zeae strain RIA 482	NR_037122.1	99.00
		L. casei strain NBRC 15883	NR_113333.1	98.92
		L. rhamnosus strain NBRC 3425	NR_113332.1	98.59
20	K5-5a	*L. paracasei* strain NBRC158889	NR_113337.1	99.91
		L. zeae strain RIA 482	NR_037122.1	99.09
		L. casei strain NBRC 15883	NR_113333.1	99.00
		L. rhamnosus strain NBRC 3425	NR_113332.1	98.54
21	K5-11	*L. paracasei* strain NBRC 15889	NR_113337.1	99.65
		L. zeae strain RIA 482	NR_037122.1	99.47
		L. casei strain NBRC 15883	NR_113333.1	99.47
		L. rhamnosus strain NBRC 3425	NR_113332.1	99.47
22	*K5-14	*L. zeae strain* RIA 482	NR_037122.1	99.21
		L. paracasei strain NBRC 15889	NR_113337.1	99.30
		L. casei strain NBRC 15883	NR_113333.1	99.21
23	*K5-15	*L. zeae* strain RIA 482	NR_037122.1	98.71
		L. paracasei strain NBRC 15889	NR_113337.1	98.79
		L. casei 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	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_041054.1	97.08
		L. zeae strain RIA 482	NR_037122.1	96.06
		L. casei strain NBRC 15883	NR_113333.1	95.96
25	*K6-3a	*L. helveticus* strain NBRC 15019	NR_113719.1	99.22
		L. gallinarum strain ATCC 33199	NR_042111.1	99.39
		L. acidophilus strain NBRC 13951	NR_113638.1	98.09
26	K6-6a	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_0410541.1	99.52
		L. zeae strain RIA 482	NR_037122.1	98.45
		L. casei strain NBRC 15883	NR_113333.1	98.36
27	K6-7	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_0410541.1	99.42
		L. zeae strain RIA 482	NR_037122.1	98.44
		L. casei strain NBRC 15883	NR_113333.1	98.36
28	K6-9a	*L. paracasei* subsp. *tolerans* strain NBRC 15906	NR_0410541.1	99.98
		L. zeae strain RIA 482	NR_037122.1	98.04
		L. casei 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	*L. paracasei* subsp. tolerans strain ATCC 25302	NR_117987.1	99.25
				98.41
		L. zeae strain RIA 482	NR_037122.1	98.23
		L. casei strain NBRC 15883	NR_113333.1	
30	*K6-14	*L. rhamnosus* strain NBRC 3425	NR_113332.1	99.45
		L. paracasei subsp. *tolerans* strain NBRC 15906	NR_0410541.1	99.54
		L. zeae strain RIA 482	NR_037122.1	99.45
		L. casei 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        CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG
k6-3a           CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG
L.gallin        CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG
                ******************************************* ****************

L.helvet        GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
k6-3a           GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
L.gallin        GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA
                **************************************** *******************
```

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)

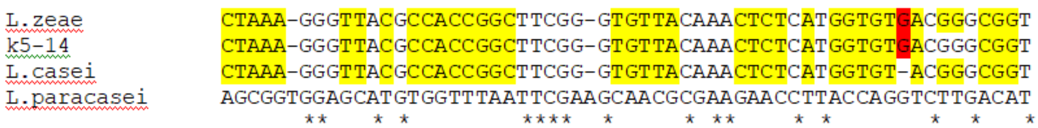


Figure 3.24 The alignment of the 16S rRNA gene of K5-14 isolate with bases of *L. zeae*, *L. casei,* and *L. paracsei*.

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.

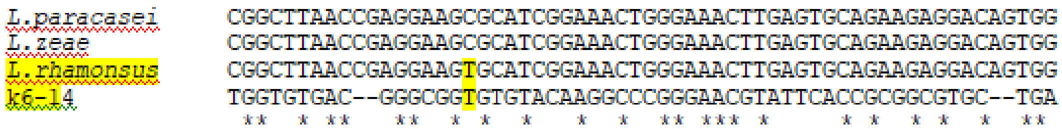


Figure 3.25 The alignment of the 16S rRNA gene of K6-14 isolate with bases of *L. zeae*, *L. rhamnosus* and *L. paracsei*.

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.

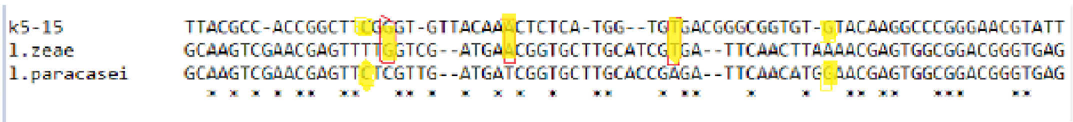


Figure 3.26 The alignment of the 16S rRNA gene of K5-15 isolate with bases of *L. zeae*, *L. casei*, and *L. paracsei*.

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 directionsof 16S rRNA sequencing
K2-3	*L. gallinarum*	*L. intestinalis*	*L. gallinarum*
K5-14	*L.paracasei*	*L.zeae*	*L.paracasei*
K5-15	*L.paracasei*	*L.zeae* and\or *L.paracasei*	*L.paracasei*
K6-3a	*L. gallinarum*	*L.helveticus*	*L.helveticus*
K6-14	*L.paracasei*	*L. rhamnosus*	*L.paracasei*

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.

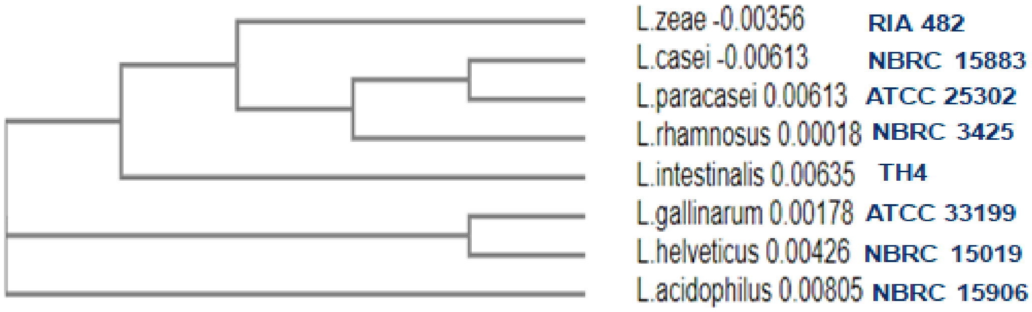


Figure 3.27 Phylogenetic tree of aligned 16S rRNA gene sequences for the known species.

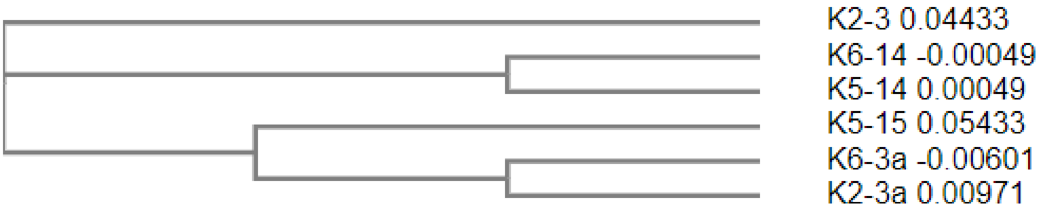


Figure 3.28 Phylogenetic tree of aligned 16S rRNA gene sequences for some isolates.

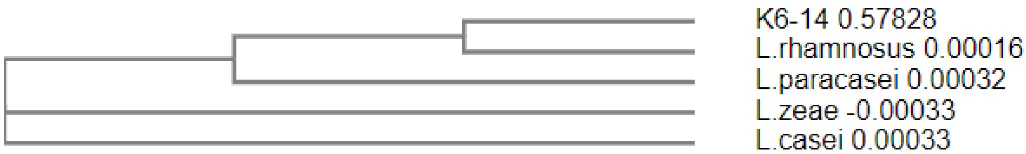


Figure 3.29 Phylogenetic tree of aligned 16S rRNA gene sequences for K6-14 with related known species.

In fact, It is also hard to differentiate *L. paracasei* subsp. *tolerans* strain NBRC 15906, *L. casei* strain NBRC 15883, and *L. zeae* 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. zeae* , 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 identifed 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 confırmed 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. kefiri* 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	*L. paracasei* subsp. *tolerans* 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	*L. gallinarum* strain ATCC 33199	99.19-99.45%	dairy products	Tamime A Y. et al, 1005
*K2-3	*L. intestinalis* strain TH4	98.27%	-	-
K4-6a, *K6-3a	*L. helveticus* strain NBRC 15019	99.23-99.66%	Bulgarian Brazilan 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	*L. zeae strain* RIA 482	99.21-99.71	-	-
*K6-14	*L. rhamnosus* 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

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

Homemade kefir from Istanbul (K5) had *L. kefiri, L. kefiranofaciens, L. acidophilus, L. delbrueckii* subsp. *bulgaricus, L. casei, L. paracasei, Saccharomyces cerevisiae, Streptocccus 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. kefiri, L. kefiranofaciens, L. delbrueckii* subsp. *bulgaricus, L. casei, L. paracasei, Saccharomyces cerevisiae, Streptocccus 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 Argentine 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 commenrcial 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. zeae* 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

Abdulamir 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 proteolyticactivity of yoghurt starters in whole milk from camels andcows". 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 kefiranofaciens ZW3 isolated from Tibet kefir" Part II. Food Hydrofoil. 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., Karbancıoglu- 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 acdid 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., & Abdulamir, 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 gatstrointestinal 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 breasted 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 kefiri 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* Ml 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 Ml 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 kefiran 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. Techno!. 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 Zanoni,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 Carlioz. (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* groupA3 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.,andHe,G.(2012)." Investigationon culturable microflora in Tibetan kefir grains from different areas of China". J. FoodSci. 77, 425–433.

Gao, J.,Gu, F.,He,J., Xiao, J.,C hen, Q., Ruan, H.,et al.(2013a)." Metagenome analysis of bacterial diversity in Tibetan kefir grains". Eur. FoodRes.Technol. 236,549–556.

Gao,J.,Gu,F., Ruan,H.,Chen, Q., He,J.,andHe,G. (2013b) ." Induction of apoptosis of gastric cancer cells SGC 7901invitro by a cell-free fraction of Tibetan kefir". Int. DairyJ. 30, 14–18.

Garofalo,C.,Osimani, A., Milanoviˇc, 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 forthe identification of Lactobacillus kefiranofaciens in microbial consortia present in kefir grains" FoodMicrobiol. 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 ScienceTechnology, 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.

Holzapfel , 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 environmentalconditions for alkaline protease production using *Bacillus subtillus* in submerged fermentation process". IJAVMS. Vol.4,issue 4,1o5-113.

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 Rev59:171-200.

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, LiuY-S, ShyuY-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., Faid, 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 kefiranofaciens* 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 kefiri 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. Crevecoeur, 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 apositive influence on blood lipoprotein profiles and inflammatory cytokines" Anopen-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 0. Kandler. (1980) "Heterogeneity of the species *Lactobacillus acidophilus* (Moro) Hansen and Moquot as revealed by biochemical characteristics and DNA-DNA hybridisation". Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe 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". FoodMicrobiol, 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., Radel, 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 In tracellular Me1 ting Temperature As Determined by Differential Scanning Calorimetry" . Journal of General Microbiology, 134, 1185-1 195.

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 l., 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 Ant onio 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 anti42 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 Irmler, 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). " Biocompatability study of *Lactobacillus casei* isolated from cucumber and evaluartion 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-Pachecoa, María Arévalo-Villenaa, Antonio Bevilacquab, Maria Rosaria Corbob, Ana Briones Péreza (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). "Rabid identification of diary 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 eith reference to lactic acid bacteria and med 22, 42.

Salminen, S., A. Ouwehand, Y. Benno, Y.K. Lee. (1999). "Probiotics: how should they bedefined? "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 Lumenal Microflora in the Gastrointestinal Tract "MicroecologyTerminology, 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. , Başyiğit 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 zeae* 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, Holzapfel 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 Puhan, Z. (1999). "Role of selected yeasts in cheese ripening: An evaluation in foil wrapped Raclette cheese". Lebensmıttel-Wıssenschaft & Technologıe (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 kefirs with natural probiotic". LWT-Food Science and Technology, 37(6), 663-667.

Yavuzdurmaz H. (2007). "Isolation, Characterizatıon, 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 Neumanna E. (2015)." Selection of lactic acid bacteria from Brazilian kefir grains for potential use as starteror probiotic cultures". Anarobe 32, 70–76.

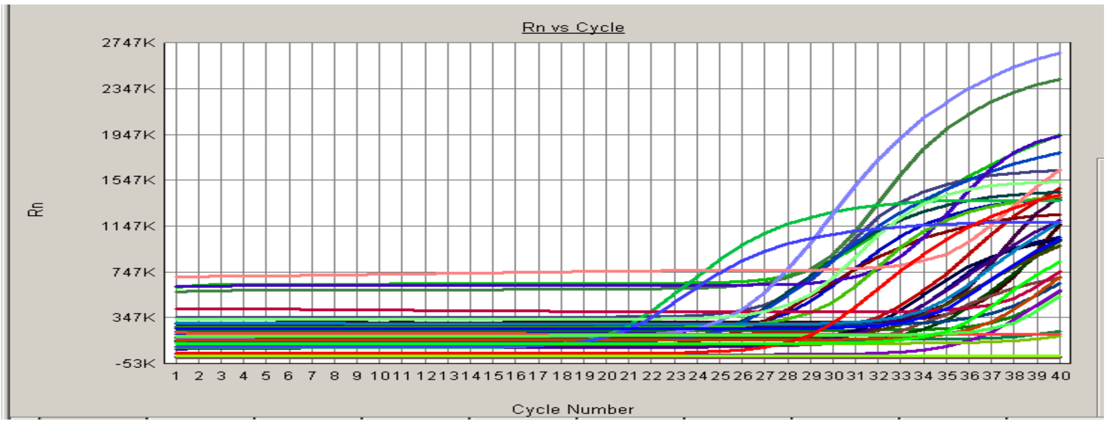
Zhou, J., X. Liu, H. Jiang, and M. Dong. (2009). "Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis". Food Microbiol. 26:770-775

Zubeyde, O., Aynur, K., & Lutfu, C. M. (2010). "Effects of Different Milk Types and Starter Cultures on Kefir " Gida, *35*(3), 177–182.

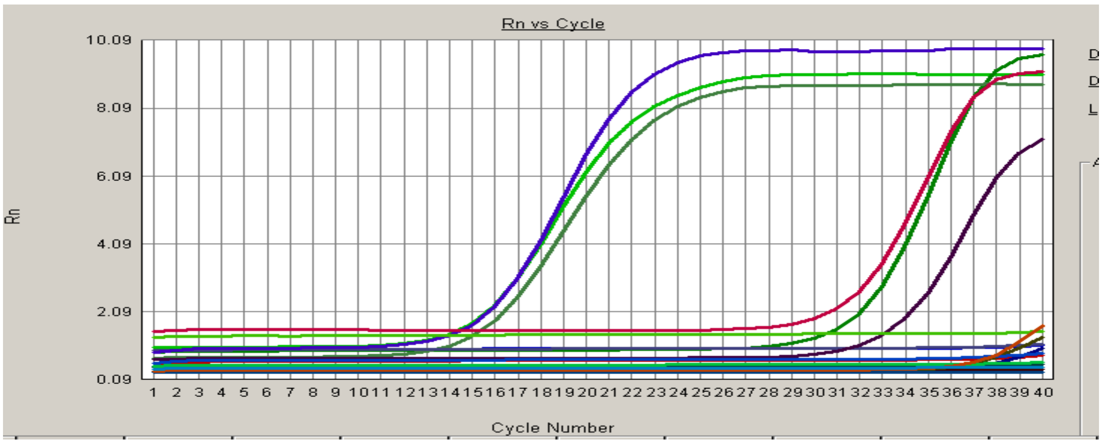
APPENDICES

APPENDIX A. Real Time PCR Results

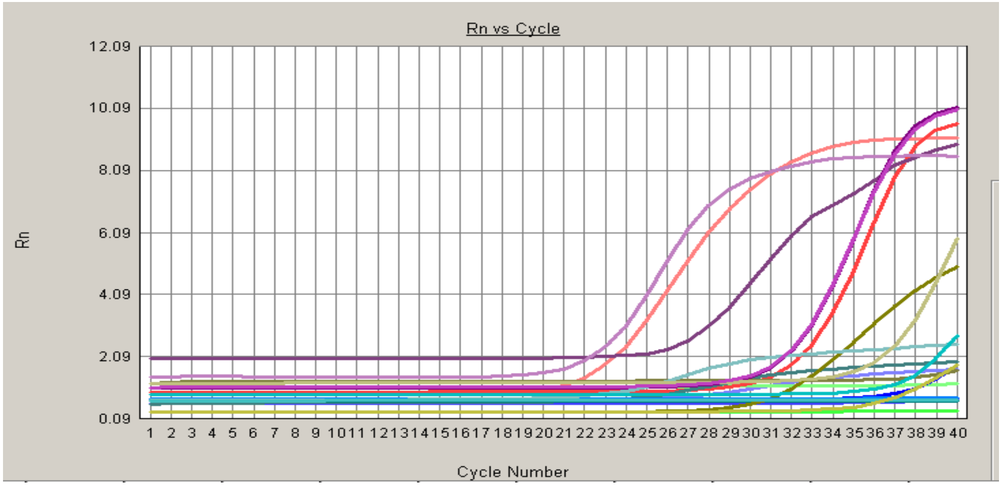
A.1 Amplification curves of kefir sample K1, K2 DNA isolate with specific primers (*L. Kefiri* , *L. acidophilus* ,*L. delbrueckii subsp. bulgaricus* ,*L. kefiranofaciens, L. fermentum* , *L. plantarum* , *S. cerevisiae* , *S. thermophilus* , *L. sobris \amylovorus*



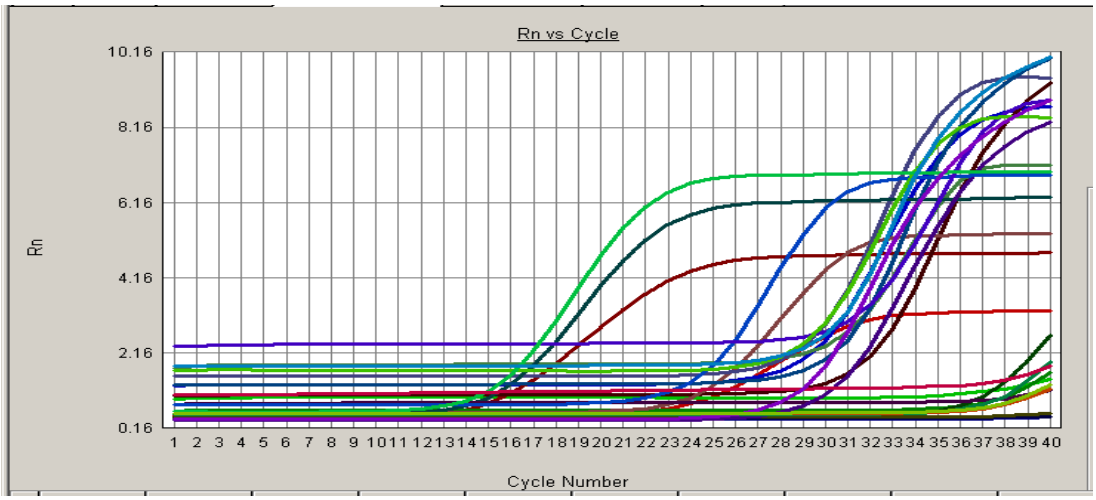
A.2 Amplification curves of kefir sample k3 DNA isolate with specific primers *(L. delbrueckii subsp. bulgaricus ,L. kefiranofaciens, L. fermentum , L. plantarum , S. cerevisiae , S. thermophilus ,L. sobris \amylovorus)*



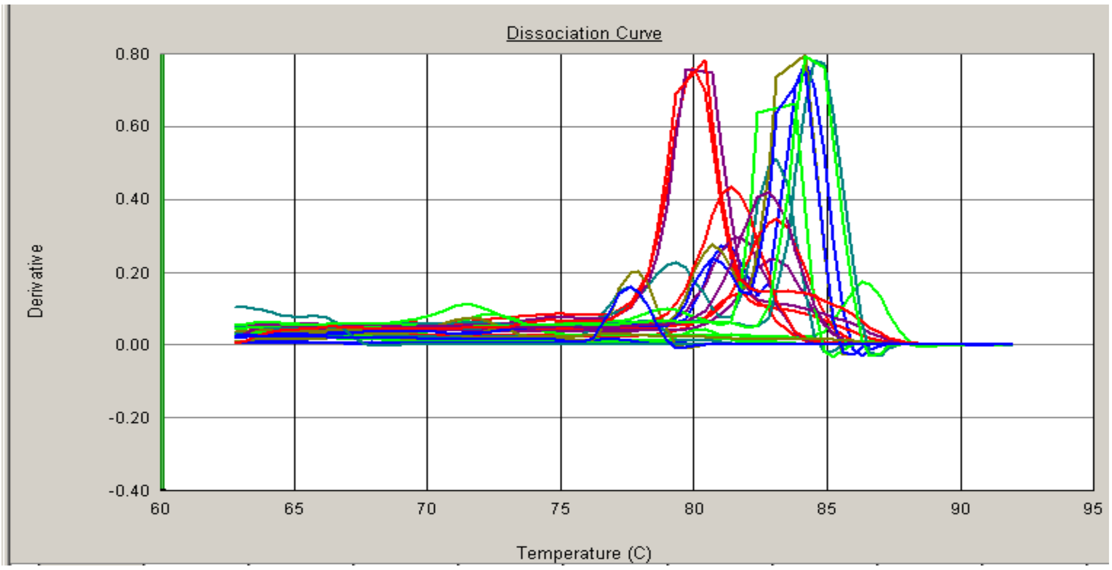
A.3 Amplification curves of kefir sample k4 DNA isolate with specific primers (*L. delbrueckii subsp. bulgaricus , L. kefiranofaciens, L. fermentum , L. plantarum , S. cerevisiae , S. thermophilus , L. sobris \amylovorus)*



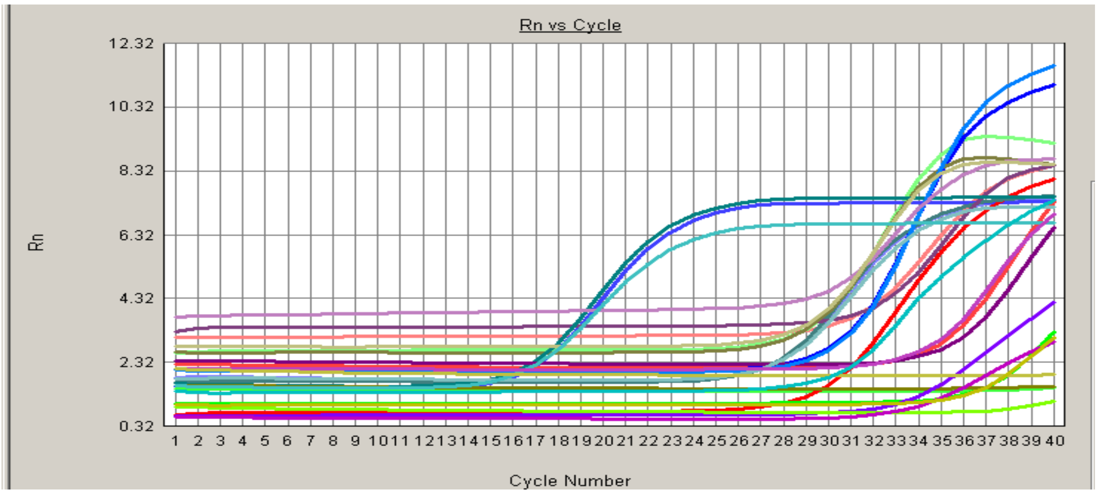
A.4 Amplification curves of kefir sample K5 DNA isolate with specific primers (*L. Kefiri , L. acidophilus , L. delbrueckii subsp. bulgaricus , L. kefiranofaciens, L. fermentum , L. plantarum , S. cerevisiae , S. thermophilus , L. sobris \amylovorus)*



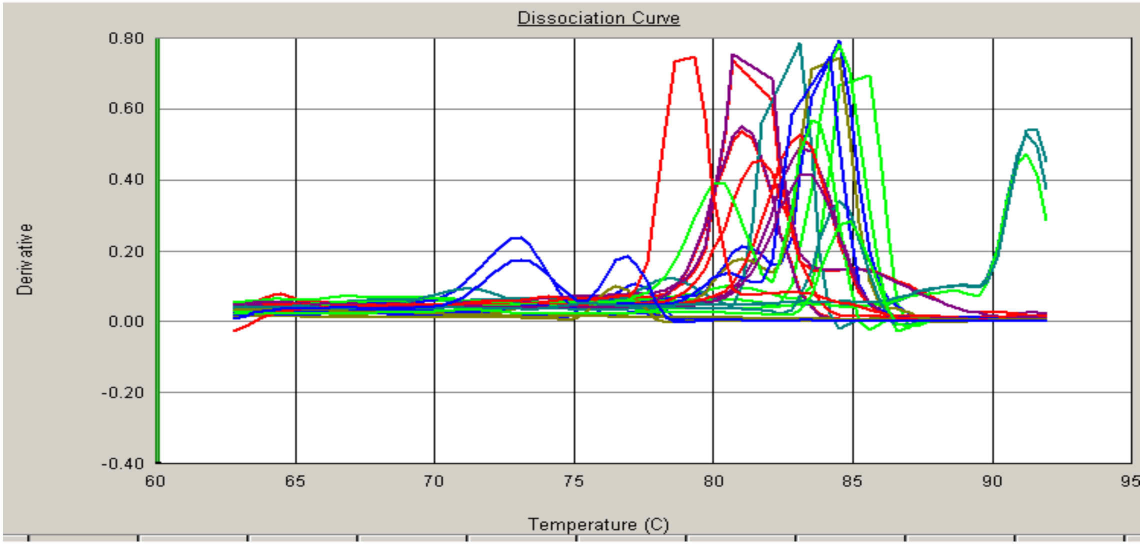
Melting curves of kefir sample (K5 and K6) DNA isolate with specific primers (*L. Kefiri* , *L. acidophilus* , *L.delbrueckii subsp. bulgaricus* , *L. kefiranofaciens, L. fermentum* , *L. plantarum* , *S. cerevisiae* , *S. thermophilus* , *L.sobris \amylovorus*



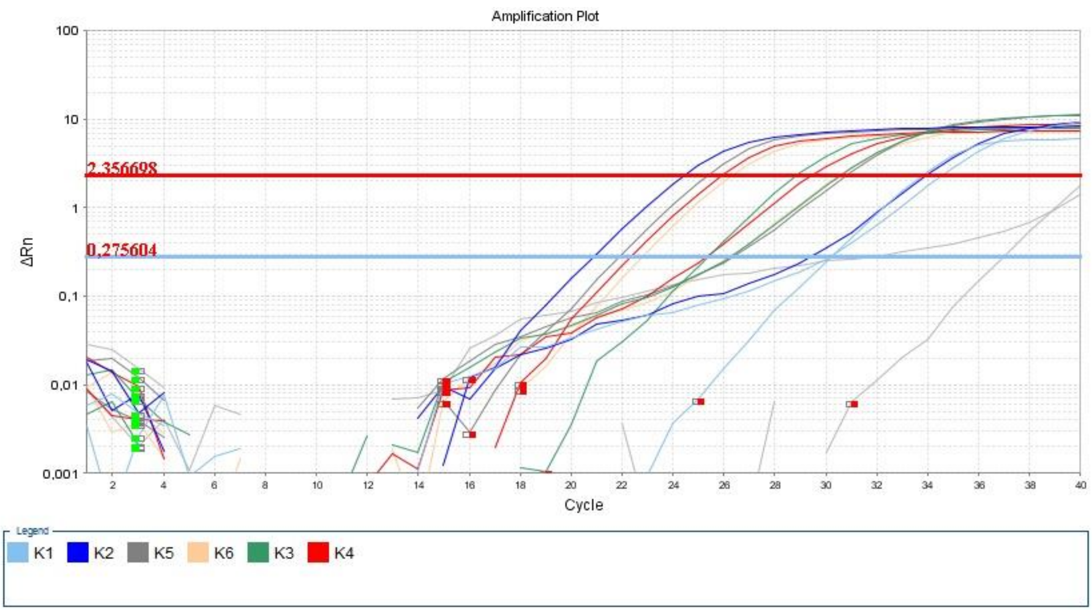
A.5 Amplification curves of kefir sample K6 DNA isolate with specific primers (L. *Kefiri* , *L.acidophilus* ,*L.delbrueckii subsp. bulgaricus* ,*L.kefiranofaciens, L.fermentum* , *L.plantarum* , *S.cerevisiae* , *S.thermophilus* ,*L.sobris \amylovorus*



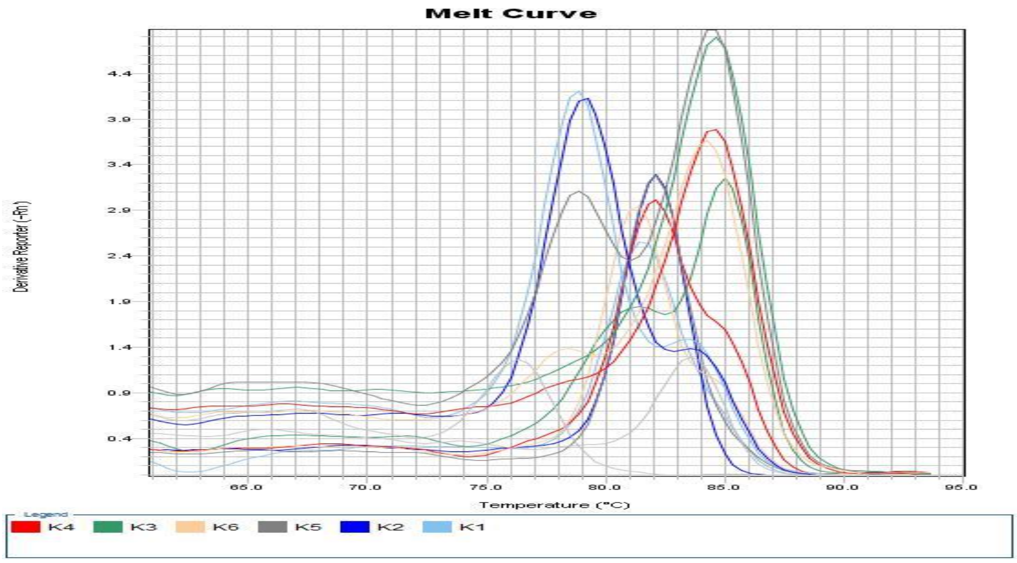
Melting curves of kefir sample K6 DNA isolate with specific primers (L. *Kefiri* , *L.acidophilus, L.delbrueckii subsp. bulgaricu ,L.kefiranofaciens, L .fermentum , L. plantarum , S.cerevisiae , S.thermophilu ,L.sobris \amylovorus*



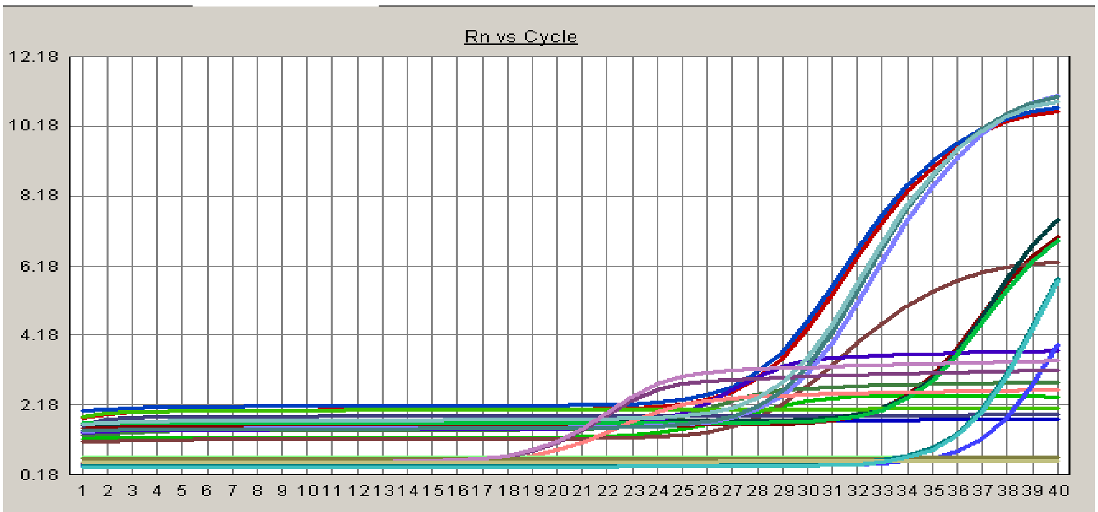
A.6 Amplification curves of kefir sample K1,K2 ,K3 , K4 ,K5, K6 DNA isolate with specific primers (L. *casei and L. paracasei)*



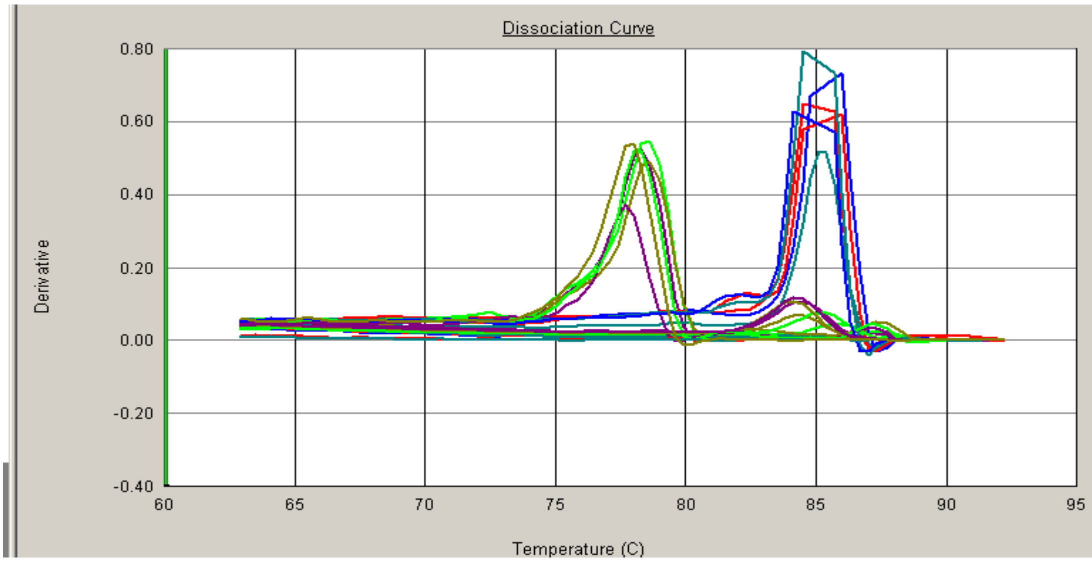
Melting curves of kefir sample K1,K2 ,K3 , K4 ,K5, K6 DNA isolate with specific primers (*L. casei and L. paracasei)*



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
GGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCAC
AATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATC
GTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGAC
GGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAAGAA
TAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTT
TCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGA
TATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAG
GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAA
ACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCAT
TAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACC
TTACCAGGTCTTGACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGA
CGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAGTTGGGCACTC
TAATGAGACTGCCGGGGACAAACCGGAGGAAGGGGGGGATGACGCCAAGCCACTT
GCCCCTATGACCGGGGCAACC

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

GGCTCCTTCCGAAGGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATGG
TGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTTCT
GATCCGCGATTACTAGCGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGC
AGTCCGAACTGAGAACAGCTTTCAGAGATTCGCTTGCCTTCGCAGGCTCGCT
TCTCGTTGTACTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCA
TGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCA
TTAGAGTGCCCAACTTAATGCTGGCAACTAATAATAAGGGTTGCGCTCGTT
GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACC
ACCTGTCTTAGCGTCCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAG
ATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCT
CCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTC
GTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGG
AAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGACTACCAGGGTA
TCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACC
AGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCG
CTACACATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCG
ATGCAATTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTCCGC
CTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTAT
TACCGCGGCTGCTGGCACGTATTTAGCCGTGACTTTCTGGTTGATTACCGTC
AAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACCAACAACAGAGCTTTA
CGATCCAAAACCTTCTTCACTCAGGCGGCGTTGCTCCATCAAACTTGCGTCC
ATTGTGGAAGATTCCCTACTGCTGCCCT

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

ACCTGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCT
GGGGACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCAT
AGTCTAGGATACCACTTGGAAACAGGTGCTAATACCGGATAATAAAGCAGA
TCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCC
CCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATG
CATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGCCC
AAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTC
TGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCT
GTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAA
TCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA
GGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAA
GAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAA
ACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGT
GGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGT
CTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATA
CCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCC
GCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGA
CCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA
TCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAG
GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAGTTGGGCACTCTAA
TGAGACTGCCGGTGACAAACCGGAGGAAGGGGG

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

ATGCAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAA
CATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAA
GTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC
CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGT
AAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC
ATGCCCCTTATGACCTGGGCTACCACGTGCTACATGGATGGTACAACGAGT
TGCGAGACCGCGAGGTCAGCTAATCCTTAAAGCCTTTCCAGTTCGGACTGT
AGGCTGCACTCGCCTACCCAAATCGGAATCCCTAGTA

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

GGCTCCTTCCCGAAGGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATG
GTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTTC
TGATCCGCGATTACTAGCGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGC
AGTCCGAACTGAGAACAGCTTTCAGAGATTCGCTTGCCTTCGCAGGCTCGCT
TCTCGTTGTACTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCA
TGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCA
TTAGAGTGCCCAACTTAATGCTGGCAACTAATAATAAGGGTTGCGCTCGTT
GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACC
ACCTGTCTTAGCGTCCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAG
ATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCT
CCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTC
GTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGG
AAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGACTACCAGGGTA
TCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACC
AGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCG
CTACACATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCG
ATGCAATTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTCCGC
CTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTAT
TACCGCGGCTGCTGGCACGTATTTAGCCGTGACTTTCTGGTTGATTACCGTC
AAATAAAGGCCAGTTACTACCTCTATCCTTCTTCCCAACAACAAAACTTTAC
AATCCAAAAACCTTCTTCACTCAGGCGGCGTTGCTCCCTCAAACTTGCGTCC
ATTGGGGAAAAATTCCCTACTGCTT

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

GGCTCCTTCCCGAAGGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATG
GTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTTC
TGATCCGCGATTACTAGCGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGC
AGTCCGAACTGAGAACAGCTTTCAGAGATTCGCTTGCCTTCGCAGGCTCGCT
TCTCGTTGTACTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCA
TGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCA
TTAGAGTGCCCAACTTAATGCTGGCAACTAATAATAAGGGTTGCGCTCGTT
GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACC
ACCTGTCTTAGCGTCCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAG
ATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCT
CCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTC
GTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGG
AAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGACTACCAGGGTA
TCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACC
AGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCG
CTACACATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCG
ATGCAATTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTCCGC
CTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTAT
TACCGCGGCTGCTGGCACGTATTTAGCCGTGACTTTCTGGTTGATTACCGTC
AAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACCAACAACAAAGCTTTA
CGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCCATCAAACTTGCGTC
CATGGGGAAGATTCCCTACTGCAC

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

ACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGA
GTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATA
ACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTC
TTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTAT
TAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAAC
TGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA
ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAG
AAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
GTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTG
ATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACT
TGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA
GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGA
CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGT
CCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGT
GCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTT
GAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGATCA
CCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGG
TTGTCGTCAGCTCGTGTCCTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTTTAGTAAGACGGCCG
GTGACAAACCGGAGGAAGGGGGGGATGACGTCGG

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

GAGTTGATCCTGGATCAGAGAAGACCCAACAAAGTTACCTCAGAAATGACG
CTGGGGACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCC
ATAGTCTAGGATACCACTTGGAAACAGGTGCTAATACCGGATAATAAAGCA
GATCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGG
CCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGA
TGCATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGC
CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAG
TCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCT
CTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGT
AATCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
TAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGG
AAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGG
AAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCG
GTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTG
GTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGA
TACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTT
CCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTAC
GACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGA
CATCTAGTGCCATCCTAAGAGATTAGGAGTTCTCTTCGGGGACGCTAAGAC
AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC
CGCAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAGTTGGGCACTCT
AATGAGACTGCCGGTGACAAACCGGAGGAAAGGGGGGATGACGTCAAGT
CATCCGGCCCC

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

TGCAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAAC
ATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAG
TGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACC
GCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGT
AAGACTGCCGGTGACAACCGGAGGAAGGTGGGGATGACGTCAATCATCAT
GCCCCTTATGACCTGGGCTACCACGTGCTACATGGATGGTACACGAGTTGC
GAGACGCGAGGTCAGCTAATCCTTAAAGCCTTTCCAGTTCGGCTGTAGGCT
GCACCCCCTAACAAATCGGA

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

AGTGTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGT
GGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAAC
ATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTT
GGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTA
GCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAACTG
AGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAAC
GCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAA
GAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAG
CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT
TATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGAT
GTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTG
AGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGA
TATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACG
CTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCC
ATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGC
CGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGATCACCT
GAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGTTG
TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCTTATGACTAGTTGCCAGCATTTAGTTGGGGCACTCTAGTAAGAACTGCCG
GGTGACAAACCGGGAAGGAAGGTGGGGATGACGTCCAAATCCTCTTGCCC
CCTTTAGGAACCTGGGGCTAACCACCGTGCTAACAAGGGAAGGGTAAAA

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

GCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGT
GCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCT
ACAGTCCGAACTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTC
GCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGG
GGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGT
CTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT
CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG
CACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CATATTACCGCGGCTGCTGGCACGTAATTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCAACAACAATCA

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

GAGTGTCTCGGTGATGATCGGTGCTTGCACCGAGATTCAACATGGGAACGA
GTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATA
ACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTC
TTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTAT
TAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAAC
TGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA
ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAG
AAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
GTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTG
ATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACT
TGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA
GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGA
CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGT
CCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAT

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

TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAA
CATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAA
GTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC
CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGT
AAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC
ATGCCCCTTATGACCTGGGCTACCACGTGCTACATGGATGGTACAACGAGT
TGCGAGACCCGAGGTCAGCTAATCCTTAAGCCATTCCAATTCGGATGTAGG
CTGAAC

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

TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGG
GACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGT
CTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGATCG
CATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCG
CGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATGCAT
AGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGT
TGTTGGTGAAGAAGGATAGAGGCAGTAACTGGCCTTTATTTGACGGTAATC
AACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAAGA
ATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTGCATCGGAAAC
TGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGG
AATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCT
GCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCG
CCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGG
TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAAT
GAAACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATC
AGCCCCTTAGGACCTGGGCTACCCCGTGCTACATGGGAAAGTACACCGGAA
GCGAGCCTGG

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

GCTTGCACCGAGATTCAACATGGAACGAGTGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAAT
ACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGC
TATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGC
TCACCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTG
GGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCT
TTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTG
TTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAG
CAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTA
AAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAAC
CGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTG
GAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTG
GCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGG
TAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGC
TAGGTGTTGGAGGGTTTCCGCCCTTCATTGCCGCATACTAACGCATAAGCGT
TCCACCTGGGCGGTTC

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

GCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCAT
GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTG
CTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTA
CAGTCCGAACTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTCG
CAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGG
GCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC
TTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCTC
GTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGC
ACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCTCCAACAACAGA
GTTTTACGACCCGAAAGCCTTCTTCCTCACGCGGCGTTGCTCCATCAAACTT
GCGTCCATTGGGGATG

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

GCAGTCGACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACAT
GGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTG
GGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGC
ATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGC
GGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTA
GCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAC
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT
GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTG
TTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAA
CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
AAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTG
GGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAA
ATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTG
TAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCC
TGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCC
CTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCG
CAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTT
TTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAA
GACTGCCGGTGACAAACCGGAGGAAGGGGGGATGACGTCAAATCATCAT
GCCCCTTATGACTGGGGCTACCCACGTGCCTACAATGGATGGTACAACCA
AGTTGCCAGAACCGCCGAGGTCAAGCTAATCTCTTAA

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

TGCAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAAC
ATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAG
TGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACC
GCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGT
AAGACTGCCGGTGACAAACCGGAGGAAGGTGGGATGACGTCAATCATCAT
GCCCCTTATGACCTGGGCTACCACGTGCTACATGGATGGTACACGAGTTGC
GAGACCCCAGGTCAGCTAATCCTTAAGCCTTTCCAGTTCGGACGTAGGCTG
CACTCCCTACCAAGTCGGATCGCAGTATCCGGATCAC

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

GCCTAATACATGCAAGTCGAACGAGTTCTTGTTGATGATCGGTGCTTGCACC
GAGATTCAACATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAAC
CTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAG
ATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTT
GGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGG
CGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGA
CACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGA
CGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGT
AAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGT
GACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGC
AGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCG
CATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATG
TGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCG
GCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACA
GGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTG
GAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGG
GGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG
GTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAA
AATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGG
GCACTCTAGTAAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACG
TCAAATATCATGCCCCTTATGACCTGGGCTACCCCCGTGCTACAATGGAGG
GACAACGAGTTGCAGACCGGGAGTCAAGCTAATCTTTAAGGCCTTTTTAGT
TCGGACTGGAGGGTGGAACTGGC

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

TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAA
CATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAA
GTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC
CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGAC

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

GGCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGT
GCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCT
ACAGTCCGAACTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTC
GCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGG
GGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGT
CTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT
CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG
CACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCTCCAACAACAGA
GTTTTACGACCCGAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCAAACT
TGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAATTTGGGCC
GGGTCTCATTCCCAATGTGGCCAATCAACCTTCAATTCGGCTACGTATCACC
GCCTTGGTGAACCCTTACCTCCCCACCAAGCTAAACCCCCCGGGTTCCATCC
AAAGCGATAACTTACCCCTTCTTTACCCAC

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

GCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCAT
GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTG
CTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTA
CAGTCCGAACTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTCG
CAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGG
GCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC
TTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCTC
GTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGC
ACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCTCCAACAACAGA
GTTTTACAACCCCAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCAAACT
TGCGTCCATTGTGGAAGAATCCCTACTGCTGCCTCCCGTAGGATTTGGGCCG
GGTCCCACTCCCAAGGGGGCCAATCAACCTCCCAGTTCGGCG

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

GGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTC
ATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCG
TGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCC
TACAGTCCGAACTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCT
CGCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAG
GGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAG
TCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCAT
GCACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATC
AAAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCA
CATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTT
GCGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAA
GGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATGGACTACC
AGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA
CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATT
TCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAG
TTTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAA
AACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCT
ACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGAT
ACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCTTCTCCAACAACAG
AGTTTTACGACCCGAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCAGAC
TTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCCTAAGAATTTGGGC
CGTGTCTCAGTCCCAATGTGGCCGATCAACCTCTCAATTCGGCTACATATCA
TCCCCTTGGTGAACCCTTACCTCCCCACTAGCTTAAACCCCCGGGTCCCTCC
AAAAGGAATGCTTACGCCTCTTTTACCCAAAAACATGGCGGGTTTCTGGATT
TTGCGGTTTAACCTCTTTTTCCAAGTTATCCCCCCTTAGGGGG

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

GCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAAC
ATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAG
TGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACC
GCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAAGACAGTGGAACTCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAAGCGGCTGTCTGGTC
TGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAATGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCCAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCCACAACCGGTGGA
GCATGTGGTTTAATTCAAGCAACCCGAGAAACCTTACCAGGGCTTGACTTCT
TTTGATCACCTGAAAAATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGGG
GGGCATGGTTGTCCTCCACCCCCGTCCGGGAAATTTGGGTTAATTCCCCCAC
CAAGGCAACCCTTTTAACAATTGCCGCCATTTATTTGGGCCCTCTTTAAAAA
TGCCGGGGAAAACCCGAGGAAGGGGGGTAAAGTCAAAATCCTTGGCCCC
TTTAAACCGGGGTCACCCCGGTCCACTGGAGGGGAAACCAAGTTTGGGAAC
CCGGGGTAAGCTAATTCTTTAAGGCCTTTCCCTTTGGGAGGGGGGGGCAC
CCCCCTCCCAAAACGGGAAATCTGGTAATTCGGAAAAAACCCCCCGGGGAA
AATTTCCCG

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

TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGG
GACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGT
CTAGGATACCACTTGGAAACAGGTGCTAATACCGGATAATAAAGCAGATCG
CATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCG
CGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATGCAT
AGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGT
TGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATC
AACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAAGA
ATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAAC
TGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGG
AATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCT
GCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCG
CCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGG
TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAGTTGGGCACTCTAAT
GAGACTGCCGGTGACAAACCGGAGGAAGGGGGGGATGACCTCAAGCATCA
TGCCCCTT

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

TCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGA
ACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGG
GATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATG
GTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGC
GTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCC
GAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCC
TACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA
GCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTG
GAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCA
GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAG
TCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGA
AACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGC
GTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC
TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT
AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTC
AGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAG
GTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGA
TCACCTGAAAGATCAGGTTTCCCCTTCGGGGGCAAATGACAGGGGGTGCAT
GGTTGCCGTCAGCCCGGGGCAGGAGAGTTTGGGGTTAAGTCCTGAACTG

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

GCAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACA
TGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGT
GGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCG
CATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGC
GGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTA
GCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAC
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT
GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTG
TTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAA
CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
AAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTG
GGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAA
ATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTG
TAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCC
TGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCC
CTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCG
CAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTT
TTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
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
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGT
AAGACTGCCGGTGACAACCGGAAGGAAGGTGGGGATGACGTCAAATCATC
ATGCCCCTTATGACCTGG

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

TGCTTGCACCGAGATTCAACATGGGAACGAGTGGCGGACGGGTGAGTAACA
CGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAA
TACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAG
CTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACG
GCTCACCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACAT
TGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATC
TTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAG
GCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAA
CTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGC
CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGC
GTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTT
AACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA
GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCA
GTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCAT
GGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAA
TGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAG
CATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAA
GAACCTTACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCC
CTTCGGGGGCAAAATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATGACTAGTTG

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

GGCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGT
GCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCT
ACAGTCCGAACTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTC
GCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGG
GGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGT
CTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT
CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG
CACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA
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	Accession
Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2089	2089	100%	0.0	99.22%	NR_042111.1
Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	2089	2089	100%	0.0	99.22%	NR_113261.1
Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2089	2089	100%	0.0	99.22%	NR_117061.1
Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	2078	2078	100%	0.0	99.05%	NR_113719.1
Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	2078	2078	100%	0.0	99.05%	NR_117060.1
Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	2078	2078	100%	0.0	99.05%	NR_042439.1
Lactobacillus acidophilus strain NBRC 13951 16S ribosomal RNA, partial sequence	2006	2006	100%	0.0	97.93%	NR_113638.1
Lactobacillus acidophilus strain VPI 6032 16S ribosomal RNA, partial sequence	2006	2006	100%	0.0	97.93%	NR_117062.1
Lactobacillus acidophilus strain JCM 1132 16S ribosomal RNA, partial sequence	2006	2006	100%	0.0	97.93%	NR_117812.1
Lactobacillus acidophilus strain BCRC10695 16S ribosomal RNA, partial sequence	2006	2006	100%	0.0	97.93%	NR_043182.1

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    CGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGCGGCGGAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  45   CGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGCGGCGGAT  104

Query  61   GGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTAGGATACCACTTGGAAACAGGTGC  120
            |||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||
Sbjct  105  GGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTGGGATACCACTTGGAAACAGGTGC  164

Query  121  TAATACCGGATAATAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCG  180
            ||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  165  TAATACCGGATAAGAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCG  224

Query  181  CTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  225  CTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAAT  284

Query  241  GATGCATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGCCCAAACT  300
            |||||||||||||||||||||||||| |||||||||||||||||||||||||||||||||
Sbjct  285  GATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACT  344

Query  301  CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  345  CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGC  404

Query  361  CGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  405  CGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAG  464

Query  421  GTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  465  GTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCA  524
```

```
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  465       GTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCA  524

Query  481       GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGC  540
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  525       GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGC  584

Query  541       GCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGA  600
                 |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  585       GCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTGCATCGGA  644

Query  601       AACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCG  660
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  645       AACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCG  704

Query  661       TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAG  720
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  705       TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAG  764

Query  721       GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGAT  780
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  765       GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGAT  824

Query  781       GAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTC  840
                 ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  825       GAGTGCTAAGTGTTGGGAGGTTTCCGCTTCTCAGTGCTGCAGCTAACGCATTAAGCACTC  884

Query  841       CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAG  900
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  885       CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAG  944

Query  901       CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT  960
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  945       CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT  1004

Query  961       AGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGTGCATGGC  1020
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1005      AGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGTGCATGGC  1064

Query  1021      TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATT  1080
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  1065      TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTT  1124
```


```
Query  1021      TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATT  1080
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  1065      TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTT  1124
```


```
Query  1081      ATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGGGACAAACCGGAGGAA  1140
                 |||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  1125      ATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAA  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 kitasatonis 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          GTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATGGTGTGACGGGCGGTGT-------   53
l.intest      --TAGGTGGCAAGCGTTG-----TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAA   53
l.gallin      --TAGGTGGCAAGCGTTG-----TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAA   53
helvit        --TAGGTGGCAAGCGTTG-----TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAA   53
                ****   *  ** ***     * * ** **    ** ** * * * *

k2-3          --------GTACAAGGCCCGGGAACGTATTCACCGCGGCGTTCTGAT--CCGCGATTACT   103
l.intest      AGATAAGTCTGATGTGAAAGCCCCCGGCTTAACCGAGGAATTGCATCGGAAACTGTGTTT   113
l.gallin      GAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTGCATCGGAAACTGTTTTT   113
helvit        GAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTGCATCGGAAACTGTTTTT   113
                       *     *   *    **  ** **** **   *          *  *   *

k2-3          AGCGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTC   163
l.intest      CTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATG   173
l.gallin      CTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATG   173
helvit        CTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGGAATGCGTAGATATATG   173
                 ** * ***    *   *** **  * **           * * ** * * *  * *

k2-3          AGAG-ATTCGCTTGCCTTCGCAGGCTCGCTTCTCGTTGT--ACTGCC--CATTGTAGCAC   218
l.intest      GAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGC   233
l.gallin      GAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGC   233
helvit        GAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGC   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 ▲ Pr

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    GGCCCGGGAACGTATTCACCGCGGCGTTCTGATCCGCGATTACTAGCGATTCCAGCTTCG  133
             ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  1300  GGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCCAGCTTCG  1241

Query  134   TGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTCAGAGATTCGCTTGCCT  193
             ||||||||||||||||||||||||||||||||||||||||||| |||||| |||||||||
Sbjct  1240  TGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTAAGAGATCCGCTTGCCT  1181

Query  194   TCGCAGGCTCGCTTCTCGTTGTACTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAG  253
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1180  TCGCAGGCTCGCTTCTCGTTGTACTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAG  1121

Query  254   GGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCATTA  313
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1120  GGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCATTA  1061

Query  314   GAGTGCCCAACTTAATGCTGGCAACTAATAATAAGGGTTGCGCTCGTTGCGGGACTTAAC  373
             ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  1060  GAGTGCCCAACTTAATGCTGGCAACTAATAACAAGGGTTGCGCTCGTTGCGGGACTTAAC  1001

Query  374   CCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTCTTAGCGTCCCCGAA  433
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1000  CCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTCTTAGCGTCCCCGAA  941

Query  434   GGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGTAAGGTTCTTCGCGT  493
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  940   GGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGTAAGGTTCTTCGCGT  881
```

```
Sbjct  940   GGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGTAAGGTTCTTCGCGT  881

Query  494   TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGT  553
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  880   TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGT  821

Query  554   TTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAG  613
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  820   TTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAG  761

Query  614   AGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGACTACCAGGGTATCT  673
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  760   AGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGACTACCAGGGTATCT  701

Query  674   AATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGAGAGCCGCC  733
             |||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||
Sbjct  700   AATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCC  641

Query  734   TTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCACT  793
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  640   TTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCACT  581

Query  794   CTCCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAATTCCTCGGTTAAGCCGAGGGC  853
             |||||||||||||||||||||| ||||||||||||||||||||||||||||||| |||||
Sbjct  580   CTCCTCTTCTGCACTCAAGAAACACAGTTTCCGATGCAATTCCTCGGTTAAGCCGGGGGC  521

Query  854   TTTCACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAAC  913
             |||||||||||||||||  |||||||||||||||||||||||||||||||||||||||||
Sbjct  520   TTTCACATCAGACTTATCTTTCCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAAC  461

Query  914   GCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTATTTAGCCGTGACTTTCTGGTTG  973
             |||||||||||||||||||||||||||||||||||||| |||||||||||||||||||||
Sbjct  460   GCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGGTTG  401

Query  974   ATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACCAACAACAGAGCTTT  1033
             ||||||||||||||  ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  400   ATTACCGTCAAATAGGGGCCAGTTACTACCTCTATCCTTCTTCACCAACAACAGAGCTTT  341

Query  1034  ACGATCC-AAAACCTTCTTCACTCAGGCGGCGTTGCTCCATCAAACTTGCGTCCATTGTG  1092
             ||||||| ||||||||||||||||| |||||||||||||||||  ||| || ||||||||
Sbjct  340   ACGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTG  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    TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  38   TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGC  97

Query  61   GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTAGGATACCACTTGGAAA  120
            ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  98   GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTGGGATACCACTTGGAAA  157

Query  121  CAGGTGCTAATACCGGATAATAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAA  180
            |||||||||||||||||||| |||||||||||||||||||||||||||||||||||||||
Sbjct  158  CAGGTGCTAATACCGGATAAGAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAA  217

Query  181  GCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  218  GCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA  277

Query  241  AGGCAATGATGCATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGC  300
            |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  278  AGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGC  337

Query  301  CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  338  CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA  397

Query  361  GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  398  GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG  457

Query  421  GATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  458  GATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTA  517

Query  481  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  518  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  577
```

```
Query  541   AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTG  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  578   AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTG  637

Query  601   CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  638   CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG  697

Query  661   GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  698   GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA  757

Query  721   CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  758   CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT  817

Query  781   AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA  840
             |||||||||||||||||||||||||||||||||| |||||||||||||||||||||||||
Sbjct  818   AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCTTCTCAGTGCTGCAGCTAACGCATTA  877

Query  841   AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  878   AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  937

Query  901   GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  938   GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT  997

Query  961   GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGT  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  998   GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGT  1057

Query  1021  GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1058  GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC  1117

Query  1081  CCTTATTATTA  1091
             |||| ||||||
Sbjct  1118  CCTTGTTATTA  1128
```

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 ▲ Previ

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   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  148

Query  120  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  208

Query  180  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  268

Query  240  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  299
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  328

Query  300  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  388

Query  360  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  419
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  448

Query  420  AATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  479
            ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  449  AATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  508

Query  480  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  539
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  568

Query  540  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  628

Query  600  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  659
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  688

Query  660  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  719
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  748

Query  720  TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  779
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749  TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  808
```

```
Query  780   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  839
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  809   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  868

Query  840   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  928

Query  900   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  929   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  988

Query  960   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1019
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  989   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1048

Query  1020  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1079
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1049  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1108

Query  1080  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1139
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1109  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1168

Query  1140  ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTAC-CAC  1198
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  1169  ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC  1228

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

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    GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTTCTGATCCGCGATTACTAG  120
             ||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  1407  GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAG  1348

Query  121   CGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTCAG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1347  CGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTCAG  1288

Query  181   AGATTCGCTTGCCTTCGCAGGCTCGCTTCTCGTTGTACTGCCCATTGTAGCACGTGTGTA  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1287  AGATTCGCTTGCCTTCGCAGGCTCGCTTCTCGTTGTACTGCCCATTGTAGCACGTGTGTA  1228

Query  241   GCCCAGGTCATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCAC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1227  GCCCAGGTCATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCAC  1168

Query  301   CGGCAGTCTCATTAGAGTGCCCAACTTAATGCTGGCAACTAATAATAAGGGTTGCGCTCG  360
             ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  1167  CGGCAGTCTCATTAGAGTGCCCAACTTAATGCTGGCAACTAATAACAAGGGTTGCGCTCG  1108

Query  361   TTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTC  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1107  TTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTC  1048

Query  421   TTAGCGTCCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGT  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1047  TTAGCGTCCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGT  988

Query  481   AAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGT  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  987   AAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGT  928

Query  541   CAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTA  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  927   CAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTA  868

Query  601   GCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGA  660
             ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  867   GCTGCAGCACTGAGAAGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGA  808

Query  661   CTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAG  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  807   CTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAG  748

Query  721   ACCAGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACA  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  747   ACCAGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACA  688
```

```
Query  781   CATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAATTCCTCG  840
             |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  687   CATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAGTTCCTCG  628

Query  841   GTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAA  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  627   GTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAA  568

Query  901   TAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTATTTAGCCG  960
             |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  567   TAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCG  508

Query  961   TGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTC-CC  1019
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  507   TGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACC  448

Query  1020  AACAACAAAACTTTACAATCCAAAAACCTTCTTCACTC  1057
             ||||||| | |||||| |||| ||||||||||||||||
Sbjct  447   AACAACAGAGCTTTACGATCCGAAAACCTTCTTCACTC  410
```

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    GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTTCTGATCCGCGATTACTAG  120
             ||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  1407  GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAG  1348

Query  121   CGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTCAG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1347  CGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTCAG  1288

Query  181   AGATTCGCTTGCCTTCGCAGGCTCGCTTCTCGTTGTACTGCCCATTGTAGCACGTGTGTA  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1287  AGATTCGCTTGCCTTCGCAGGCTCGCTTCTCGTTGTACTGCCCATTGTAGCACGTGTGTA  1228

Query  241   GCCCAGGTCATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCAC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1227  GCCCAGGTCATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCAC  1168

Query  301   CGGCAGTCTCATTAGAGTGCCCAACTTAATGCTGGCAACTAATAATAAGGGTTGCGCTCG  360
             ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  1167  CGGCAGTCTCATTAGAGTGCCCAACTTAATGCTGGCAACTAATAACAAGGGTTGCGCTCG  1108

Query  361   TTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTC  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1107  TTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTC  1048

Query  421   TTAGCGTCCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGT  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1047  TTAGCGTCCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGT  988

Query  481   AAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGT  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  987   AAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGT  928

Query  541   CAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTA  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  927   CAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTA  868

Query  601   GCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGA  660
             ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  867   GCTGCAGCACTGAGAAGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGA  808

Query  661   CTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAG  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  807   CTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAG  748

Query  721   ACCAGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACA  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  747   ACCAGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACA  688
```

```
Query  781   CATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAATTCCTCG  840
             |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  687   CATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAGTTCCTCG  628

Query  841   GTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAA  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  627   GTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAA  568

Query  901   TAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTATTTAGCCG  960
             |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  567   TAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCG  508

Query  961   TGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACC  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  507   TGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACC  448

Query  1021  AACAACAAAGCTTTACGATCCGAAAACCT  1049
             ||||||| |||||||||||||||||||||
Sbjct  447   AACAACAGAGCTTTACGATCCGAAAACCT  419
```

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

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	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	1956	1956	100%	0.0	98.64%	NR_037122.1
☑	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1953	1953	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

See 1 more title(s)

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   GGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  99   GGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGC  158

Query  121  TAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  159  TAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCG  218

Query  181  CTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  219  CTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGAT  278

Query  241  GATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  279  GATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACT  338

Query  301  CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  339  CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGC  398

Query  361  CGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  399  CGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGC  458

Query  421  AGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCA  480
            ||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  459  AGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCA  518

Query  481  GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  519  GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGC  578

Query  541  GCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  579  GCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGA  638

Query  601  AACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCG  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  639  AACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCG  698

Query  661  TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAG  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  699  TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAG  758
```

```
Query  721   GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGAT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  759   GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGAT  818

Query  781   GAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTC  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  819   GAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTC  878

Query  841   CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAG  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  879   CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAG  938

Query  901   CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  939   CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT  998

Query  961   TTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGT  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  999   TTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGT  1058

Query  1021  TGTCGTCAGCTCGTGTCCTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATG  1080
             ||||||||||||||||| ||| ||||||||||||||||||||||||||||||||||||||
Sbjct  1059  TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATG  1118

Query  1081  ACTAGTTGCCAGCATTTAGTTGG  1103
             |||||||||||||||||||||||
Sbjct  1119  ACTAGTTGCCAGCATTTAGTTGG  1141
```

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

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	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_117061.1
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_117060.1
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_113638.1
Lactobacillus acidophilus strain VPI 6032 16S ribosomal RNA, partial sequence	1945	1945	99%	0.0	98.20%	NR_117062.1
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    AATGACGCTGGGGACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATA  63
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  77   AATGACGCTGGGGACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATA  136

Query  64   GTCTAGGATACCACTTGGAAACAGGTGCTAATACCGGATAATAAAGCAGATCGCATGATC  123
            |||| ||||||||||||||||||||||||||||||||||||| |||||||||||||||||
Sbjct  137  GTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGATCGCATGATC  196

Query  124  AGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGT  183
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  197  AGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGT  256

Query  184  TGGTAAGGTAACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACTGAACGGCC  243
            |||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  257  TGGTAAGGTAACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCC  316

Query  244  ACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCAC  303
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  317  ACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCAC  376

Query  304  AATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAA  363
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  377  AATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAA  436

Query  364  GCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAA  423
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  437  GCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAA  496

Query  424  CCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT  483
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  497  CCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT  556

Query  484  GTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCC  543
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  557  GTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCC  616

Query  544  CTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGT  603
            |||||||||||||||||| |||||||||||||||||||||||||||||||||||||||||
Sbjct  617  CTCGGCTTAACCGAGGAACTGCATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGT  676

Query  604  GGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGC  663
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  677  GGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGC  736

Query  664  GGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGA  723
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  737  GGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGA  796

Query  724  TACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTC  783
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  797  TACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCTTCTC  856
```

```
Query  784   AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT  843
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  857   AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT  916

Query  844   CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG  903
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  917   CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG  976

Query  904   CGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATCCTAAGAGATTAGGAGTTCTCTTC  963
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  977   CGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTC  1036

Query  964   GGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT  1023
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1037  GGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT  1096

Query  1024  AAGTCCCGCAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAGTTGGGCACTCTAA  1083
             ||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  1097  AAGTCCCGCAACGAGCGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAA  1156

Query  1084  TGAGACTGCCGGTGACAAACCGGAGGAAAG-GGGG  1117
             |||||||||||||||||||||||||||| | ||||
Sbjct  1157  TGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGG  1191
```

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 15906 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_025880.1
☑	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	2196	2196	99%	0.0	99.42%	NR_117987.1
☑	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 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 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-AGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  59
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  29   TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  88

Query  60   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  148

Query  120  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  208

Query  180  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  268

Query  240  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  299
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  328

Query  300  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  388

Query  360  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  419
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  448

Query  420  AATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  479
            ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  449  AATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  508

Query  480  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  539
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  568

Query  540  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  628

Query  600  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  659
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  688

Query  660  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  719
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  748
```

```
Query  720   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  808

Query  780   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  839
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  809   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  868

Query  840   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  928

Query  900   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  929   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  988

Query  960   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1019
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  989   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1048

Query  1020  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1079
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1049  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1108

Query  1080  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGAC-A  1138
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  1109  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1168

Query  1139  ACCGGAGGAAGGTGGGGATGACGTC-AATCATCATGCCCCTTATGACCTGGGCTAC-CAC  1196
             ||||||||||||||||||||||||| |||||||||||||||||||||||||||||| |||
Sbjct  1169  ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC  1228

Query  1197  GTGCTACA-TGGATGGT  1212
             |||||||| ||||||||
Sbjct  1229  GTGCTACAATGGATGGT  1245
```

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

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	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_025880.1
☑	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	98.36%	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    AGTGTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGCGGACGG  60
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  42   AGT-TCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGCGGACGG  100

Query  61   GTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  101  GTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTA  160

Query  121  ATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  161  ATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCT  220

Query  181  TTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  221  TTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGA  280

Query  241  TACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  281  TACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCC  340

Query  301  TACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  341  TACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCG  400

Query  361  CGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  401  CGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAG  460

Query  421  AGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGC  480
            ||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  461  AGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGC  520

Query  481  AGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  521  AGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGC  580

Query  541  AGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  581  AGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA  640

Query  601  CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  641  CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA  700

Query  661  GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGC  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  701  GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGC  760
```

```
Query  721   TCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGA  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  761   TCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGA  820

Query  781   ATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCG  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  821   ATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCG  880

Query  841   CCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  881   CCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG  940

Query  901   GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTT  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  941   GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTT  1000

Query  961   TGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGTTG  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1001  TGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGTTG  1060

Query  1021  TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATGAC  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1061  TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATGAC  1120

Query  1081  TAGTTGCCAGCATTTAGTTGGGGCACTCTAGTAAGAACTGCCGGGTGACAAACCGGGAAG  1140
             ||||||||||||||||||| ||||||||||||||| |||||||| ||||||||||| | |
Sbjct  1121  TAGTTGCCAGCATTTAGTT-GGGCACTCTAGTAAGA-CTGCCGG-TGACAAACCGG-A-G  1175

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

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

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 strain NBRC 15889 16S ribosomal RNA, partial sequence	1794	1794	100%	0.0	99.80%	NR_113337.1
☑	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	1794	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 sequence

Sequence ID: NR_113337.1 Length: 1495 Number of Matches: 1

Range 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     GCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTGA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1463  GCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTGA  1404

Query  61    CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACT  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1403  CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACT  1344

Query  121   AGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTTA  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1343  AGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTTA  1284

Query  181   AGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1283  AGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGTG  1224

Query  241   TAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1223  TAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTC  1164

Query  301   ACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1163  ACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT  1104

Query  361   CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTG  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1103  CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTG  1044

Query  421   TCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCTG  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1043  TCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCTG  984

Query  481   GTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  983   GTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCC  924

Query  541   GTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCGT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  923   GTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCGT  864

Query  601   TAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATG  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  863   TAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATG  804

Query  661   GACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  803   GACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC  744

Query  721   AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTA  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  743   AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTA  684
```

```
Query  781    CACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCCT  840
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  683    CACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCCT  624

Query  841    CGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCCC  900
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  623    CGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCCC  564

Query  901    AATAAATCCGGATAACGCTTGCCACCTACATATTACCGCGGCTGCTGGCACGTAATTAGC  960
              ||||||||||||||||||||||||||||| |||||||||||||||||||||||| |||||
Sbjct  563    AATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGC  504

Query  961    CGTGGCTTTCTGGTTGG  977
              |||||||||||||||||
Sbjct  503    CGTGGCTTTCTGGTTGG  487
```

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

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	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	98.86%	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  AACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  179  AACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGC  238

Query  181  GTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAACTGAGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  239  GTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAACTGAGA  298

Query  241  GGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  299  GGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAG  358

Query  301  GGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  359  GGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTT  418

Query  361  TCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  419  TCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGT  478

Query  421  GACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  479  GACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG  538

Query  481  GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539  GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCT  598

Query  541  GATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  599  GATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCA  658

Query  601  GAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACC  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  659  GAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACC  718

Query  661  AGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCG  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  719  AGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCG  778

Query  721  AACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGG  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  779  AACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGG  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* 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	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 3425 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    TCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  35   TCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGC  94

Query  61   GGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95   GGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAG  154

Query  121  ATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  155  ATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCT  214

Query  181  ATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  215  ATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG  274

Query  241  CGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  275  CGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCA  334

Query  301  AACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  335  AACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA  394

Query  361  ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  395  ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGT  454

Query  421  CGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGT  480
            ||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  455  CGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGT  514

Query  481  GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  515  GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGC  574

Query  541  GAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  575  GAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCAT  634

Query  601  CGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  635  CGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA  694

Query  661  TGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGC  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  695  TGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGC  754
```

```
Query  721   TGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAA  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  755   TGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAA  814

Query  781   CGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGC  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  815   CGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGC  874

Query  841   ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  875   ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA  934

Query  901   CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  935   CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC  994

Query  961   ATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCA  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  995   ATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCA  1054

Query  1021  TGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCT  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1055  TGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCT  1114

Query  1081  TATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAAACCGGA  1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1115  TATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAAACCGGA  1174

Query  1141  GGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTA  1189
             |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1175  GGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTA  1223
```

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	99.66%	NR_113719.1
☑	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.66%	NR_042111.1
☑	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.66%	NR_113261.1
☑	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.66%	NR_117061.1
☑	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.66%	NR_117060.1
☑	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.66%	NR_042439.1
☑	Lactobacillus acidophilus strain NBRC 13951 16S ribosomal RNA, partial sequence	2054	2054	100%	0.0	98.37%	NR_113638.1

```
k4-6a           CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG
helveticus      CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG
l.gallinarium   CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG
                ******************************************* ****************

k4-6a           GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
helveticus      GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
l.gallinarium   GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA
                **************************************** *******************

k4-6a           CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT
helveticus      CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT
l.gallinarium   CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT
                ************************************************************

k4-6a           AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA
helveticus      AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA
l.gallinarium   AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCTTCTCAGTGCTGCAGCTAACGCATTA
                ********************************** *************************
```

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 Mat

Score	Expect	Identities	Gaps	Strand
2137 bits(1157)	0.0	1165/1169(99%)	0/1169(0%)	Plus/Plus

```
Query  1    TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28   TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGC  87

Query  61   GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTGGGATACCACTTGGAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  88   GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTGGGATACCACTTGGAAA  147

Query  121  CAGGTGCTAATACCGGATAAGAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  148  CAGGTGCTAATACCGGATAAGAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAA  207

Query  181  GCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  208  GCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA  267

Query  241  AGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  268  AGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGC  327

Query  301  CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  328  CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA  387

Query  361  GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  388  GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG  447

Query  421  GATAGAGGCAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTA  480
            |||||||| |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448  GATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTA  507

Query  481  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  508  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  567

Query  541  AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  568  AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTG  627

Query  601  CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG  660
            ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  628  CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG  687

Query  661  GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  688  GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA  747

Query  721  CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  748  CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT  807
```

```
Query  781   AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  808   AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA  867

Query  841   AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  868   AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  927

Query  901   GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  928   GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT  987

Query  961   GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGT  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  988   GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGT  1047

Query  1021  GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1048  GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC  1107

Query  1081  CCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAAACTGCCGGTGACAAACC  1140
             |||||||||||||||||||||||||||||||||||||||||| ||||||||||| |||||
Sbjct  1108  CCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGATAAACC  1167

Query  1141  GGAGGAAGGTGGGGATGACGTCAAGTCAT  1169
             |||||||||||||||||||||||||||||
Sbjct  1168  GGAGGAAGGTGGGGATGACGTCAAGTCAT  1196
```

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

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	1447	1447	100%	0.0	99.87%	NR_041054.1
☑	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 Matc

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  TTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAACTGAGAGGT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  242  TTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAACTGAGAGGT  301

Query  241  TGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  302  TGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGA  361

Query  301  ATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  362  ATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCG  421

Query  361  GGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGAC  420
            |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  422  GGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGAC  481

Query  421  GGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  482  GGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG  541

Query  481  GCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542  GCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGAT  601

Query  541  GTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  602  GTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAA  661

Query  601  GAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGT  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  662  GAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGT  721

Query  661  GGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAAC  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  722  GGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAAC  781

Query  721  AGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTT  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  782  AGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTT  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 GenBank 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	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  GCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTGA  1404

Query  60    CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACT  119
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1403  CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACT  1344

Query  120   AGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTTA  179
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1343  AGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTTA  1284

Query  180   AGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGTG  239
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1283  AGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGTG  1224

Query  240   TAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTC  299
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1223  TAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTC  1164

Query  300   ACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT  359
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1163  ACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT  1104

Query  360   CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTG  419
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1103  CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTG  1044

Query  420   TCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCTG  479
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1043  TCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCTG  984

Query  480   GTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCC  539
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  983   GTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCC  924

Query  540   GTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCGT  599
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  923   GTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCGT  864

Query  600   TAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATG  659
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  863   TAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCATG  804

Query  660   GACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC  719
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  803   GACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC  744

Query  720   AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTA  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  743   AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTA  684
```

```
Query  780   CACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCCT  839
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  683   CACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCCT  624

Query  840   CGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCCC  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  623   CGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCCC  564

Query  900   AATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGC  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  563   AATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGC  504

Query  960   CGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCT  1019
             ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  503   CGTGGCTTTCTGGTTGGATACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCTTCT  444

Query  1020  CCAACAACAGAGTTTTACGACCCGAAAGCCTTCTT  1054
             |||||||||||||||||||||||||||||||||||
Sbjct  443   CCAACAACAGAGTTTTACGACCCGAAAGCCTTCTT  409
```

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

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	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-ACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  58
            ||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  29   TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  88

Query  59   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  118
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  148

Query  119  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  178
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  208

Query  179  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  238
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  268

Query  239  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  298
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  328

Query  299  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  358
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  388

Query  359  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  418
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  448

Query  419  AATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  478
            ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  449  AATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  508

Query  479  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  538
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  568

Query  539  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  598
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  628

Query  599  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  658
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  688

Query  659  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  718
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  748
```

```
Query  719   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  778
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  808

Query  779   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  838
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  809   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  868

Query  839   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  898
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  928

Query  899   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  958
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  929   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  988

Query  959   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1018
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  989   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1048

Query  1019  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1078
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1049  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1108

Query  1079  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1138
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1109  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1168

Query  1139  ACCGGAGGAAgggggggATGACGTCAAATCATCATGCCCCTTATGACTGGGGCTA  1193
             |||||||||||| ||||||||||||||||||||||||||||||||||  ||||||
Sbjct  1169  ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTA  1223
```

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

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	2224	2224	100%	0.0	99.43%	NR_041054.1
Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2224	2224	100%	0.0	99.43%	NR_025880.1
Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2220	2220	100%	0.0	99.35%	NR_113337.1
Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2211	2211	99%	0.0	99.35%	NR_117987.1
Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2159	2159	100%	0.0	98.45%	NR_037122.1
Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2156	2156	100%	0.0	98.37%	NR_113333.1
Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2152	2152	100%	0.0	98.37%	NR_041893.1
Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2145	2145	100%	0.0	98.21%	NR_115534.1
Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2130	2130	100%	0.0	98.05%	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 1256 GenBank Graphics ▼ Next Match ▲ Previous M

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  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  208

Query  180  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  268

Query  240  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  299
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  328

Query  300  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  388

Query  360  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  419
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  448

Query  420  AATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  479
            ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  449  AATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  508

Query  480  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  539
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  568

Query  540  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  628

Query  600  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  659
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  688

Query  660  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  719
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  748
```

```
Query  720   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  808

Query  780   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  839
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  809   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  868

Query  840   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  928

Query  900   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  929   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  988

Query  960   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1019
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  989   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1048

Query  1020  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1079
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1049  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1108

Query  1080  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1139
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1109  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1168

Query  1140  ACCGGAGGAAGGTGGG-ATGACGTC-AATCATCATGCCCCTTATGACCTGGGCTAC-CAC  1196
             |||||||||||||||| |||||||| |||||||||||||||||||||||||||||| |||
Sbjct  1169  ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC  1228

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

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

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 strain NBRC 15889 16S ribosomal RNA, partial sequence	2206	2206	100%	0.0	99.75%	NR_113337.1
☑	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 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_025880.1
☑	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2191	2191	99%	0.0	99.67%	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    GCCTAATACATGCAAGTCGAACGAGTTCTTGTTGATGATCGGTGCTTGCACCGAGATTCA  60
            ||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  19   GCCTAATACATGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCA  78

Query  61   ACATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  79   ACATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGA  138

Query  121  TAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  139  TAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTG  198

Query  181  AAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  199  AAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGG  258

Query  241  TAACGGCTCACCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGG  300
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  259  TAANGGCTCACCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGG  318

Query  301  ACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319  ACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACG  378

Query  361  CAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379  CAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTT  438

Query  421  GTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  439  GTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAG  498

Query  481  CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  499  CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAT  558

Query  541  TTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  559  TTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTT  618

Query  601  AACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCC  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  619  AACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCC  678

Query  661  ATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCT  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  679  ATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCT  738

Query  721  GGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGG  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  739  GGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGG  798
```

```
Query  781   TAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGC  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  799   TAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGC  858

Query  841   AGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAA  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  859   AGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAA  918

Query  901   TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  919   TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC  978

Query  961   CTTACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAA  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  979   CTTACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAA  1038

Query  1021  AATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1039  AATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG  1098

Query  1081  CAACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTG  1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1099  CAACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTG  1158

Query  1141  CCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAAT-ATCATGCCCCTTATGACCTG  1199
             ||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  1159  CCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTG  1218

Query  1200  GGC  1202
             |||
Sbjct  1219  GGC  1221
```

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

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 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    TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  29   TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  88

Query  61   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  148

Query  121  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  208

Query  181  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  209  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAANGGCTCA  268

Query  241  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  328

Query  301  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  388

Query  361  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  448

Query  421  AATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  449  AATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  508

Query  481  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  568

Query  541  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  628

Query  601  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  688

Query  661  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  748

Query  721  TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749  TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  808
```

```
Query  781   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  809   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  868

Query  841   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  928

Query  901   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  929   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  988

Query  961   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  989   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1048

Query  1021  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1049  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1108

Query  1081  AACCCTTATGACTAGTTG  1098
             ||||||||||||||||||
Sbjct  1109  AACCCTTATGACTAGTTG  1126
```

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

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 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_117987.1
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 15906 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    ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTAC  119
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1404  ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTAC  1345

Query  120   TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTT  179
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1344  TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTT  1285

Query  180   AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT  239
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1284  AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT  1225

Query  240   GTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT  299
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1224  GTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT  1165

Query  300   CACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC  359
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1164  CACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC  1105

Query  360   TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT  419
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1104  TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT  1045

Query  420   GTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT  479
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1044  GTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT  985

Query  480   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  539
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  984   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  925

Query  540   CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCG  599
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  924   CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCG  865

Query  600   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  659
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  864   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  805

Query  660   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  719
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  804   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  745

Query  720   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  744   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  685
```

```
Query  780   ACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCC  839
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  684   ACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCC  625

Query  840   TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  624   TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC  565

Query  900   CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  564   CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG  505

Query  960   CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTC  1019
             |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  504   CCGTGGCTTTCTGGTTGGATACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCTTC  445

Query  1020  TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCA  1079
             |||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||
Sbjct  444   TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCACGCGGCGTTGCTCCATCA  385

Query  1080  AACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAATTTGGGCCG  1136
              ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  384   GACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCG  328
```

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

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 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

```
l.zeae          GCGGCGTGCCTAATACATGCAAGTCGAACGAGTTTTGGTCGATGAACGGTGCTTGCATCG
l.paracasei     GCGGCGTGCCTAATACATGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCG
k5-14           --GGCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTG-TTACAAACTCTCATGGTG
                  ***  **    ** * *        **  * *   *  * * *      **      *
```

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   TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTT  179
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1344  TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTT  1285

Query  180   AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT  239
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1284  AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT  1225

Query  240   GTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT  299
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1224  GTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT  1165

Query  300   CACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC  359
             ||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  1164  CACCGGCAGTCTTACTAGAGTGCCCAACTRAATGCTGGCAACTAGTCATAAGGGTTGCGC  1105

Query  360   TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT  419
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1104  TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT  1045

Query  420   GTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT  479
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1044  GTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT  985

Query  480   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  539
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  984   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  925

Query  540   CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCG  599
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  924   CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCG  865

Query  600   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  659
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  864   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  805

Query  660   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  719
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  804   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  745

Query  720   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  744   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  685
```

```
Query  780   ACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCC  839
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  684   ACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCC  625

Query  840   TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  624   TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC  565

Query  900   CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  564   CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG  505

Query  960   CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTC  1019
             |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  504   CCGTGGCTTTCTGGTTGGATACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCTTC  445

Query  1020  TCCAACAACAGAGTTTTACAACCCCAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCA  1079
             ||||||||||||||||||| |||| ||||||||||||||||| |||||||||||||||||
Sbjct  444   TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCACGCGGCGTTGCTCCATCA  385

Query  1080  AACTTGCGTCCATTGTGGAAGAATCCCTACTGCTGCCTCCCGTAGGA-TTTGGGC  1133
              ||||||||||||||||||||| |||||||||||||||||||||||| |||||||
Sbjct  384   GACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGC  330
```

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

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 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

```
k5-15          TTACGCC-ACCGGCTTCGGGT-GTTACAAACTCTCA-TGG--TGTGACGGGCGGTGT-GTACAAGGCCCGGGAACGTATT
l.zeae         GCAAGTCGAACGAGTTTTGGTCG--ATGAACGGTGCTTGCATCGTGA--TTCAACTTAAAACGAGTGGCGGACGGGTGAG
l.paracasei    GCAAGTCGAACGAGTTCTCGTTG--ATGATCGGTGCTTGCACCGAGA--TTCAACATGCAACGAGTGGCGGACGGGTGAG
                 * * * * **  **   ** *  *  *  *  *   **    * **    *    *   ** **   ***    **
```

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    ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTAC  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1404  ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTAC  1345

Query  121   TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTT  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1344  TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTT  1285

Query  181   AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1284  AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT  1225

Query  241   GTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1224  GTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT  1165

Query  301   CACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC  360
             ||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  1164  CACCGGCAGTCTTACTAGAGTGCCCAACTRAATGCTGGCAACTAGTCATAAGGGTTGCGC  1105

Query  361   TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1104  TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT  1045

Query  421   GTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1044  GTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT  985

Query  481   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  984   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  925

Query  541   CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCG  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  924   CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCG  865

Query  601   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  864   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  805

Query  661   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  804   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  745

Query  721   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  744   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  685
```

```
Query  781   ACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCC  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  684   ACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCC  625

Query  841   TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  624   TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC  565

Query  901   CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  564   CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG  505

Query  961   CCGTGGCTTTCTGGTTGGATACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCTTC  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  504   CCGTGGCTTTCTGGTTGGATACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCTTC  445

Query  1021  TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCA  1080
             |||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||
Sbjct  444   TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCACGCGGCGTTGCTCCATCA  385

Query  1081  GACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCCTAAGAATTTGGGCCGTGT  1140
             ||||||||||||||||||||||||||||||||||||||||| || || ||||||||||||
Sbjct  384   GACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGT  325

Query  1141  CTCAGTCCCAATGTGGCCGATCAACCTCTCAATTCGGCTACATATCATCCCCTTGGTGAA  1200
             ||||||||||||||||||||||||||||||| ||||||||| ||||||| ||||||||| 
Sbjct  324   CTCAGTCCCAATGTGGCCGATCAACCTCTCAGTTCGGCTACGTATCATCGCCTTGGTGAG  265

Query  1201  CCCTTACCTCCCCA-CTAGCTTAAACCCC-CGGGTCC  1235
             || ||||||| ||| |||||| | || || |||||||
Sbjct  264   CCGTTACCTCACCAACTAGCTAATACGCCGCGGGTCC  228
```

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

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	1832	1832	99%	0.0	97.06%	NR_041054.1
Lactobacillus paracasei strain R094 16S ribosomal RNA gene, 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	95.96%	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 ▲ Previou

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   CGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  87   CGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTT  146

Query  121  GGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  147  GGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGG  206

Query  181  CGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  207  CGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCT  266

Query  241  CACCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  267  CACCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGAC  326

Query  301  ACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  327  ACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG  386

Query  361  ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387  ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGA  446

Query  421  AGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCT  480
            ||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  447  AGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCT  506

Query  481  AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  507  AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGG  566

Query  541  CGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  567  CGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGG  626

Query  601  AAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAAGACAGTGGAACTCCATGTGTAG  660
            ||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  627  AAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAG  686

Query  661  CGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAAGCGGCTGTCTGGTCTGTA  720
            ||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  687  CGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTA  746
```

```
Query  721   ACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCAT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  747   ACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCAT  806

Query  781   GCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAATGCCGCAGCTAACG  840
             ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  807   GCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACG  866

Query  841   CATTAAGCATTCCGCCTGGGGAGTACGACCGCCAGGTTGAAACTCAAAGGAATTGACGGG  900
             |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  867   CATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGG  926

Query  901   GGCCCCCACAACCGGTGGAGCATGTGGTTTAATTC-AAGCAACCCGAGAAACCTTACCAG  959
             ||||| ||||| ||||||||||||||||||||||| ||||||| |||  |||||||||||
Sbjct  927   GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG  986

Query  960   GGCTTGACTTCTTTTGATCACCTGAAAAATCAGGTTTCCCCTTCGGGGGCAAAATGACAg  1019
             | |||||| |||||||||||||||| | ||||||||||||||||||||||||||||||||
Sbjct  987   GTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAG  1046

Query  1020  ggggggCATGGTTGTCCTCCACCCCCGTCCGGGAAAT-TTGGGTTAATTCCCCCACCAAG  1078
             | || ||||||||||| ||  | |  ||| | || || ||||||||| |||| || | ||
Sbjct  1047  GTGGTGCATGGTTGTCGTCAGCTCGTGTC-GTGAGATGTTGGGTTAAGTCCCGCAACGAG  1105

Query  1079  -GCAACCCTT  1087
              |||||||||
Sbjct  1106  CGCAACCCTT  1115
```

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

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	1766	1766	100%	0.0	99.49%	NR_042111.1
Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	1766	1766	100%	0.0	99.49%	NR_113261.1
Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1766	1766	100%	0.0	99.49%	NR_117061.1
Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	1760	1760	100%	0.0	99.38%	NR_113719.1
Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1760	1760	100%	0.0	99.38%	NR_117060.1
Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1760	1760	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_113638.1
Lactobacillus acidophilus strain VPI 6032 16S ribosomal RNA, partial sequence	1716	1716	100%	0.0	98.56%	NR_117062.1

```
l.helvat        CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG
k6-3a           CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG
l.gallinarium   CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG
                ******************************************* ****************

l.hevat         GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
k6-3a           GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
l.gallinarium   GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA
                **************************************** *******************
```

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    TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28   TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGC  87

Query  61   GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTAGGATACCACTTGGAAA  120
            ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  88   GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTGGGATACCACTTGGAAA  147

Query  121  CAGGTGCTAATACCGGATAATAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAA  180
            |||||||||||||||||||| |||||||||||||||||||||||||||||||||||||||
Sbjct  148  CAGGTGCTAATACCGGATAAGAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAA  207

Query  181  GCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  208  GCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA  267

Query  241  AGGCAATGATGCATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGC  300
            ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  268  AGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGC  327

Query  301  CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  328  CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA  387

Query  361  GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  388  GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG  447

Query  421  GATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448  GATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTA  507

Query  481  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  508  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  567

Query  541  AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  568  AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTG  627

Query  601  CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG  660
            ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  628  CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG  687

Query  661  GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA  720
            |||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct  688  GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA  747

Query  721  CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  748  CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT  807
```

```
Query  781   AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  808   AAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA  867

Query  841   AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  868   AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  927

Query  901   GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  928   GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT  987

Query  961   GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGT  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  988   GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGT  1047

Query  1021  GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1048  GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC  1107

Query  1081  CCTTATTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACC  1140
             |||| ||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  1108  CCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGATAAACC  1167

Query  1141  GGAGGAAGG-GGGG  1153
             ||||||||| ||||
Sbjct  1168  GGAGGAAGGTGGGG  1181
```

Alignment

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

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 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 15883 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 3425 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

See 1 more title(s)

Range 1: 35 to 1068 GenBank Graphics ▼ Next Match ▲ Previous Match

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   GGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95   GGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAG  154

Query  121  ATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  155  ATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCT  214

Query  181  ATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  215  ATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG  274

Query  241  CGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  275  CGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCA  334

Query  301  AACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  335  AACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA  394

Query  361  ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  395  ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGT  454

Query  421  CGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGT  480
            ||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  455  CGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGT  514

Query  481  GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  515  GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGC  574

Query  541  GAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  575  GAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCAT  634

Query  601  CGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  635  CGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA  694

Query  661  TGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGC  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  695  TGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGC  754
```

```
Query  721   TGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAA  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  755   TGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAA  814

Query  781   CGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGC  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  815   CGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGC  874

Query  841   ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  875   ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA  934

Query  901   CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  935   CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC  994

Query  961   ATCTTTTGATCACCTGAAAGATCAGGTTTCCCCTTCGGGGGC-AAATGACAGGGGGTGCA  1019
             ||||||||||||||||| |||||||||||||||||||||||| |||||||||| ||||||
Sbjct  995   ATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCA  1054

Query  1020  TGGTTGCCGTCAGC  1033
             |||||| |||||||
Sbjct  1055  TGGTTGTCGTCAGC  1068
```

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

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	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 482 16S ribosomal RNA, partial sequence	2139	2139	100%	0.0	98.44%	NR_037122.1
Lactobacillus casei strain NBRC 15883 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 M

Score	Expect	Identities	Gaps	Strand
2204 bits(1193)	0.0	1210/1217(99%)	6/1217(0%)	Plus/Plus

```
Query  1    TGC-AGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  59
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  29   TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  88

Query  60   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  148

Query  120  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  208

Query  180  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  268

Query  240  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  299
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  328

Query  300  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  388

Query  360  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  419
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  448

Query  420  AATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  479
            ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  449  AATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  508

Query  480  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  539
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  568

Query  540  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  628

Query  600  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  659
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  688

Query  660  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  719
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  748
```

```
Query  720   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  808

Query  780   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  839
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  809   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  868

Query  840   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  928

Query  900   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  929   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  988

Query  960   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1019
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  989   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1048

Query  1020  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1079
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1049  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1108

Query  1080  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGAC-A  1138
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  1109  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1168

Query  1139  ACCGGAGGAAGGTGGGGATGACGTC-AATCATCATGCCCCTTATGACC-GGGCTAC-CAC  1195
             ||||||||||||||||||||||||| |||||||||||||||||||||| ||||||| |||
Sbjct  1169  ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC  1228

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

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

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	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	97.96%	NR_113333.1
Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2204	2204	100%	0.0	97.96%	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 3425 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    TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  29   TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG  88

Query  61   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89   AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG  148

Query  121  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG  208

Query  181  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA  268

Query  241  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC  328

Query  301  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  GGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT  388

Query  361  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAG  448

Query  421  AATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  480
            |||||||||||||||||||||| |||||||||||||||||||||||||||||||||||||
Sbjct  449  AATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA  508

Query  481  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG  568

Query  541  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA  628

Query  601  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG  688

Query  661  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  GTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC  748
```

```
Query  721   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC  808

Query  781   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  809   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA  868

Query  841   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869   TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG  928

Query  901   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  929   CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT  988

Query  961   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  989   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT  1048

Query  1021  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1049  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1108

Query  1081  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1109  AACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA  1168

Query  1141  -CCGGAAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTAC-CA  1198
              |||| ||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  1169  ACCGG-AGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACA  1227

Query  1199  CGTGCTAC-ATGGATGGTACACCAA-TTGCAAAACCGCGAGGTC-AGCTAATCTCTTAAA  1255
             |||||||| |||||||||||| | | |||| | ||||||||||| |||||||||||||||
Sbjct  1228  CGTGCTACAATGGATGGTACAACGAGTTGCGAGACCGCGAGGTCAAGCTAATCTCTTAAA  1287

Query  1256  GCCTTTC-CAGTTC  1268
             ||| ||| ||||||
Sbjct  1288  GCCATTCTCAGTTC  1301
```

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

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 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	2060	2060	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 Matcl

Score	Expect	Identities	Gaps	Strand
2145 bits(1161)	0.0	1184/1193(99%)	9/1193(0%)	Plus/Plus

```
Query  1    CGAGTTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGGAACGAGTGGCGGA  60
            |||| ||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  18   CGAG-TTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACAT-GGAACGAGTGGCGGA  75

Query  61   CGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  76   CGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATG  135

Query  121  CTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  136  CTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATC  195

Query  181  GCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  196  GCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGA  255

Query  241  TGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  256  TGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAC  315

Query  301  TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  316  TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACG  375

Query  361  CCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  376  CCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGG  435

Query  421  CAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  436  CAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCC  495

Query  481  AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  496  AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAG  555

Query  541  CGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556  CGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGG  615

Query  601  AAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGC  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  616  AAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGC  675

Query  661  GTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGA  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  676  GTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGA  735

Query  721  GGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGA  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  736  GGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGA  795
```

```
Query  781   TGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATT  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  796   TGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATT  855

Query  841   CCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  856   CCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA  915

Query  901   GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  916   GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC  975

Query  961   TTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGG  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  976   TTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGG  1035

Query  1021  TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAT  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1036  TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAT  1095

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

Query  1141  GAAGGTGGGGATGAACGTCAAATCATCATGCCCCCTTATGAACCTGGGGCTA  1193
             |||||||||| ||| |||||||||||||||||||| |||||| |||||| |||
Sbjct  1154  GAAGGTGGGG-ATG-ACGTCAAATCATCATGCCCC-TTATGA-CCTGGG-CTA  1201
```

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

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 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 3425 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	99.36%	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    CGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGG
L.zeae         CGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGG
L.rhamonsus    CGGCTTAACCGAGGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGG
k6-14          TGGTGTGAC--GGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGC--TGA
                **  * **   **  * *  *   *  *  ** *** *       *  * *  *  **
```

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    ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTAC  119
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1404  ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTAC  1345

Query  120   TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTT  179
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1344  TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTT  1285

Query  180   AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT  239
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1284  AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT  1225

Query  240   GTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT  299
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1224  GTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT  1165

Query  300   CACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC  359
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1164  CACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC  1105

Query  360   TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT  419
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1104  TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT  1045

Query  420   GTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT  479
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1044  GTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT  985

Query  480   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  539
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  984   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  925

Query  540   CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCG  599
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  924   CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCG  865

Query  600   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  659
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  864   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  805

Query  660   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  719
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  804   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  745

Query  720   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  744   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  685
```

```
Query  600   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  659
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  864   TTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCATTCATCGTTTACGGCAT  805

Query  660   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  719
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  804   GGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA  745

Query  720   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  744   CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCT  685

Query  780   ACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCGCTTCC  839
             |||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  684   ACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCACTTCC  625

Query  840   TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  624   TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC  565

Query  900   CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  564   CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG  505

Query  960   CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTC  1019
             |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  504   CCGTGGCTTTCTGGTTGGATACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCTTC  445

Query  1020  TCCA-CAACAAAATTTTACGACCCGAAAGCCTTCTTCACTCACGCGGCGTTGCTCCATCA  1078
             |||| ||||| | |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  444   TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCACGCGGCGTTGCTCCATCA  385

Query  1079  GACTTGCGTCCATTG  1093
             |||||||||||||||
Sbjct  384   GACTTGCGTCCATTG  370
```

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Hassan, Manal

Nationality: Egyptian

Date and Place of Birth: 15 March 1983, Egypt

Marital Status: Married

Phone: +90 5350718060

Email: manal.hassan@metu.edu.tr

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.