REPUBLIC OF TURKEY
KARADENIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL MICROBIOLOGY

INVESTIGATION OF PROBIOTIC AND BIOSURFACTANT PROPERTIES OF *LACTOBACILLUS* ISOLATED FROM DIFFERENT FERMENTED FOODS

By
Jaleel SAMANJE

IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY IN MEDICAL MICROBIOLOGY

Prof. Dr. Ali Osman KILIÇ

TRABZON-2018

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APPROVAL

This dissertation satisfies all requirements for the degree of Doctor of Philosophy

Prof. Dr. Faruk AYDIN

Head of the Department of Medical Microbiology

The dissertation titled titled "Investigation of Probiotic and Biosurfactant Properties of *Lactobacillus* Isolated from Different Fermented Foods" prepared by Jaleel SAMANJE, at the Department of Medical Microbiology, Graduate School of Health Sciences, Karadeniz Technical University, was examined for scope and scientific quality in accordance with the Karadeniz Technical University Rules and Regulations Governing Graduate Studies and accepted as Doctoral dissertation.

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DECLARATION

I declare that this dissertation is writing in accordance with the standards of the Karadeniz Technical University, Graduate School of Health Sciences thesis writing guide. I declare that this dissertation is the result of my own and that all sources or materials used for this dissertation have been duly acknowledged. I confidently declare that this dissertation has not submitted to this or any other university for the award of any academic degree, diploma or certificate.

02.05.2018
Jaleel SAMANJE

DEDICATION

To the utmost knowledge lighthouse, to our greatest and most honoured prophet Mohammed may peace and grace from Allah be upon him.

To the beloved which settled in my heart and I looking for seeing her eagerly, Republic of Iraq.

To the soul of my lovely father who I missed so much and wish that he presents with me. To the big heart, symbole of love, softness and kindness my dearest mother for her continued love, guidance and support throughout my life. Hope she live a healthy and long life. To the source of love and inspiration in my life and career my sisters and brother.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviations	Key
ATCC	American Type Culture Collection
BHIB	Brain Heart Infusion Broth
BLAST	Basic Local Alignment Search Tool
BPB	Bromocresol Purple Broth
BP	Base Pair
CFS	Cell-Free Supernatants
CFU	Colony Forming Units
DCs	Dendritic Cells
DNA	Deoxyribonucleic Acid
DMSO	Dimethyl Sulfoxide
DEMEM	Dulbecco's Modified Eagle Medium
EFSA	The European Food Safety Authority
EI	Emulsification index
FAO	Food and Agriculture Organization
FDA	American Food and Drug Administration
FHEL	Facultatively Heterofermentative *Lactobacillus*
GALT	Gut-associated Lymphoid Tissue
GRAS	Generally Recognized as Safe
ID	Adhesion Index
IgA	Immunoglobulin Type A
IL	Interleukin
IZD	Inhibition Zone Diameters
LAB	Lactic acid bacteria
MRS	De Man, Rogosa and Sharpe agar
NK	Natural Killer Cell
NZDRI	New Zealand Dairy Research Institute
XG	Revolution Per Minutes
UTI	Urinary Tract Infection
WHO	World Health Organization

1. ABSTRACT

Investigation of Probiotic and Biosurfactant Properties of *Lactobacillus* Isolated from Different Fermented Foods

Lactic acid bacteria have been used for many years as natural bio preservatives in fermented foods with health benefits to the host. Selection of strains as a probiotic is very important which is mainly based on evaluating the physiological characteristics of the selected *Lactobacillus* strains. In the current study, a number of 103 *Lactobacillus* strains were recovered from 50 different dairy products. Phenotypic characterization of the strains indicated that all them were gram-positive, oxidase and catalase negative bacilli. The characteristic of these strains as a probiotic strain primarily were based on their tolerance to low acid and high bile salt concentration, resistance to pepsin and pancreatin at various concentrations. Finally, 14 *Lactobacillus* strains were selected for further analysis for the probiotic potentials. These tests revealed resistance to antibiotics, biosafety assessment (absence of haemolytic properties); ability to inhibit different pathogens, in vitro ability to adhere to the HT-29 cells. Based on these tests, only nine strains, TLB 504, TLB 506, TLB 509, TLB 513, TLB 514, TLB 554, TLB 580, TLB 582, TLB 590 that could have good probiotic properties were identified. While TLB 504 and TLB 590 strains were the most adherent bacteria among nine strains, all 14 strains inhibitited the pathogenic bacteria by releasing of organic acid or hydrogen peroxidase or both. The 14 strains were analyzed for their ability to produce other antimicrobial substances such as biosurfactants. Six out of the 14 *Lactobacillus* strains, TLB 504, TLB 506, TLB 509, TLB 513, TLB 582, TLB 590, were able to produce cell bounded biosurfactants. Based on Matrix Assisted Laser Desorption/Ionization, Time of Flight Mass Spectrometry (MALDI-TOF) and 16S rDNA sequence analysis all nine strains were identified as *L. plantarum* species. In conclusion, from this study potential probiotic *Lactobacillus* strains could be recovered from fermented dairy food products.

Key Words: Antimicrobial activity, *Lactobacillus*, probiotic, biosurfactant, dairy food

2. ÖZET

Farklı Gıdalardan İzole Edilen Laktobasil Türlerinin Probiyotik ve Biyosürfektan Özelliklerinin Araştırılması

Laktik asitbakterileri, fermente gıdalarda doğal biyokoruyucu olarak uzun yıllardan beri kullanılmaktadır ve ayrıca bu bakterilerin konağın sağlığı üzerine yararlı etkilerinin olduğu bilinmektedir. Bununla birlikte, bir *Laktobasillus* suşunun probiyotik olarak seçilirken suşun fizyolojik karakterizasyonunun bilinmesi önemlidir. Bu çalışmada 50 farklı süt ürününden, 103 Lactabacillussuşu izole edildi. Bu suşların fenotopik karakterizasyonu sonucunda hepsinin gram pozitif, oksidaz ve katalaz negatif basiller olduğu görüldü. Suşların probiyotik olarak değerlendirilmesinde, düşük asit ve yüksek safra tuzu konsantrasyonlarını tolere edebilme, pepsin ve pankreatine dayanıklılık gibi özelliklerine bakıldı. Sonuçta sadece probiyotik olabilme potansiyeline sahip 14 Lactobacillus suşu ileri analizlere dahil edildi. Bu testler seçilen 14 suşun antibiyotik duyarlılık profillerini, biyogüvenlik değerlendirmelerini (hemolitik özelliklerinin olmaması); çeşitli patojen bakterileri inhibe etme ve in vitro olarak HT-29 hücre hattına bağlanma yeteneklerini ortaya çıkardı. Bahsi geçen bu test sonuçlarına göre dokuz suşun (TLB 504, TLB 506, TLB 509, TLB 513, TLB 514, TLB 554, TLB 580, TLB 582 ve TLB 590) iyi probiyotik özellik gösterdiği kanısına varıldı. TLB 504 ve TLB 509 suşlarının en fazla bağlanma özelliği gösteren suşlar olarak belirlenirken, seçilen 14 suşun hepsinin, organik asit veya hidrojen peroksidaz ya da bunların her ikisini ortama salarak bakterileri inhibe ettikleri gösterildi. On dört suş, biyosürfaktan adı verilen başka bir antimikrobiyal madde üretme yetenekleri bakımından da karakterize edildi. On dört suştan altısının (TLB 582, TLB 504, TLB 506, TLB 509, TLB 513 ve TLB 590) hücre bağımlı biyosürfaktan üretebildiği tespit edildi. Matrix Assisted Laser Desorption/Ionization, Time of Flight Mass Spectrometry (MALDI-TOF) ve 16S rDNA sekans analizlerine göre dokuz suş *L. plantarum* olarak tanımlandı. Sonuç olarak bu çalışmada fermente süt ürünlerinden potansiyel probiyotik özelliği olan *Lactobacillus* suşları elde edilebildi.

Anahtar Sözcükler: Antimikrobiyal aktivite, biyosürfaktan, laktobasil, probiyotik, süt ürünleri.

3. INTRODUCTION AND AIMS

Food play a major role not only to satisfy the hunger but also maintain a good health. By consuming certain types of foods, most people believe that they can prevent disease and live longer (1). According to the Food and Agricultural Organization (FAO) and World Health Organization (WHO) experts, probiotic foods, which are the foods that contain live microorganisms, are proven to offer beneficial effects for humans when they are consumed in sufficient amounts.

A number of studies demonstrated that the probiotics which are mostly consisted of fermented foods played significant roles in health benefit. Historically, food fermentation process is regarded as the most widely used method to extend the shelf life of the food (2). Among the microorganisms involved in food fermentation, lactic acid bacteria (LAB) is one of the main group of microorganisms that are involved in food fermentation process, which is mainly due to their ability to produce many organic acids and bacteriocins. They lack of cytochrome and able to grow under aerobic condition, aerotolerant in nature, fastidious, acid tolerant and strictly fermentative. They provide their health benefits by using various mechanisms including inhibiting of growth of pathogenic bacteria and as well as helping the formation of the immune response of the hosts (3). *Lactobacillus, Lactococcus*, *Enterococcus*, *Streptococcus*, and *Bifidobacterium* are among the popular genera that are involved in food fermentation.

Based on the carbohydrate metabolism, members of the LAB are frequently categorized into two groups; the homofermentatives which produce lactic acid from carbon. Species such as *Streptococcus, Enterococcus, Pediococcus, Lactococcus*, and some lactococci are some examples that are categorized in homofermentatives. The heterofermentative group including *Weissella*, *Leuconostoc*, and some lactobacilli produce lactate as well as CO_2, ethanol or acetate from glucose by utilizing the phosphoketolase pathway (4).

A number of criteria were set for the selection of probiotic strains by WHO, FAO, and the European Food Safety Authority (EFSA). Origin of strains, genetic makeup and their physiological features are some of the critical points for selections. In addition to these, the probiotics should promote health benefits to its host, should not carry any genes responsible for antibiotic resistance transmission, should withstand the transit through the gastrointestinal tract, should remain viable during the storage for a longer period, and

competitiveness with respect to microbial species inhabiting the intestinal ecosystem (5). Among the LAB, the *Lactobacillus* is one the important members that involve in the microbiota of humans and other vertebrates, and they are also the most bacteria that contribute to the fermentation of different foods which may enhance the quality and safety of food (6). They have the ability to adapt varied selective pressure both inside and outside of the intestinal environment with their theirmetabilic activities, growth rate and carbohydrate metabolism.

The increasing of multidrug-resistant strains and the development of biofilm formation resulted in declining the effectiveness of the antibiotic treatments against pathogenic bacteria. Probiotics have been reported as one of the strategies to combat the growing antibiotic resistance and biofilm infections (7). The antibacterial actions of probiotic lactobacilli, mostly species/strain specific, and they can also act against pathogenic bacteria present in the gastrointestinal tract just by releasing some substances having antimicrobial properties such as H_2O_2, lactic acid, organic acids and bacteriocins (8). In addition, the probiotics compite with many other pathogenic agents for nutrients and spaces so that they can hinder the spreading of pathogenic agents within the body (9).

For characterization of lactobacilli as probiotic, the acid resistance property is one of the important criteria, and it has been reported that the pH ranges up to 3 can be accepted as a standard value for a given strain to be probiotics (10). The acid resistance appears to be varying among probiotic lactobacilli. In two independent studies, survival of *L. plantarum* obtained from olives survived at pH 2.0 and 3.0 for about 2-3 h, and *L. animals* at pH of 2.0 for 4 h duration have been reported (11, 12).

One of the features that probiotics strains that delivered through food system should first survive during the transit from the gastrointestinal tract to provide its beneficial effects in the host gut after attaching and surviving in the small intestine (13). Normally, with the use of some specific foods and a type of delivery systems, the acid sensitive strains can be buffered in the stomach. Tolerance to bile salt is another an important issue for given probiotics, and usually from 0.15 to 0.3 % concentration was proposed as a selecting criterion of probiotics to be used for humans (14). *L. fermentum* and *L. acidophilus* recovered from buffalo milk were demonstrated resistence of bile salt in a range of 0.05 up to 0.3 % (15).

The adherence ability of *Lactobacillus* strains to the intestinal mucosal surfaces is another key factor for selection of probiotic strains. The more adherent ability in the mucosal surface is more likely for a probiotic strain to colonize in the gut and resist the flow of the gut that may clear the non-attached ones. In most cases, the probiotic *Lactobacillus* strains can prevent the adhesion of the pathogenic bacteria to the intestine or it can displace them from the area. For instance, it has been reported that *E. coli* and *S. typhimurim* strains were prevented from adhering to colorectal adenocarcinoma cell 2 (Caco-2) an in *vitro* setting by some *Lactobacillus* strains including *L. casei* Shirota ACA-DC 6002, *L. plantarum* ACA-DC 146, and *L. paraccasei* subsp. *tolerans* ACA-DC 403 (16).

In dairy fermentation, bacteriophage infection can render the bacterial growth and metabolic activities. Particularly, when a phage infects the host bacteria at the early stage of fermentation, it may result in insufficient lactic acid production resulting in high pH and high residual lactose concentrations (17). Several investigations have also demonstrated that different strains of the LAB are able to synthesize surface-active molecules namely biosurfactants (18). The increased interest in the biosurfactant production from *Lactobacillus* strains are due to their antibacterial activity and their application in the food industry. Investigating the existence of microorganisms in certain foods and identification of the strains are prerequisites for being employed in the potential industrial application as a bio-preservative and probiotic product.

The aim of this study was to isolate and characterize *Lactobacillus* spp. from both homemade and commercial fermented products and investigate their probiotic properties.

4. LITERATURE REVIEW

4.1. Definition and History of Probiotics

Probiotics can be described as microorganism used as food components or dietary supplement that support good health for the host (19). ”Let food be the medicine and medicine be the food” the age-old saying by Hippocrates, therefore microbial cultures such as bacteria and yeast have been used for many years by mixing them with food (20). In 1908, Ellie Metchnikoff recommended that human being should consume fermented milk containing lactobacilli for longer and healthier lives (21). The age longitivity investigation was performed on Bulgarian peasants who had consumed a large quantity of sour milk. After this, many investigators start to examine the beneficial effects of lactic acid bacteria in both human and animals (22).

4.2. Microorganisms Applied in Probiotic Products

The LAB is the major group of bacteria that are used both in food fermentation and as food preservatives. Among this group, the genus *Lactobacillus* are the most widely used strains as probiotics that can be applied to the starter cultures to stimulate the flavor and texture foods, and also provide some nutritional value to some dairy products, fermented plant and meat (23). Like *Lactobacillus*, *Bifidobacterium* is also the most frequently applied as well as *Streptococcus, Propionibacterium, Bacillus, Enterococcus* and yeasts in the food industry (24).

4.3. The General Actions of Probiotics on Public Health

There have been many investigations regarding the health benefits of probiotics. Benefits of some specific probiotics strains in the public health were widely characterized and established, and even clinically well documented. These benefits include lowering of cholesterol, stimulation of immune systems, decreasing of cancer cells, act as antimicrobial effects, prevention of some infections like urogenital and *Helicobacter pylori* infections, reducing of allergic symptoms, and involving in mineral metabolism are among the many (25).

4.3.1. Probiotics and Lactose Intolerance

Most people, commonly non-Caucasian suffer from lactose intolerance who cannot digest the lactose in dairy products due to the absence of an essential enzymes, β-galactosidase. The symptoms of lactose intolerant appear when consuming the milk or the

products that containing lactose. Bloating, flatulence, abdominal pain, and diarrhea are some of the major symptoms related to the lactose intolerant (26). The probiotics promote its beneficial effects on lactose intolerance are because of the lower lactose concentration and the high lactase activity of the bacterial preparation used in the production (27).

4.3.2. Effect of Probiotics Immune System

Although the effects of probiotic strains on the immune system are not well understood, several researchers documented *in vitro* and in vivo studies of the positive and stimulating effects of probiotic microorganisms on immune system stimulation (28, 29). Pretreatment of the mixture with *Lactobacillus* strains leads to stimulating the phagocytosis and bactericidal activity against different pathogens agents like *S. aureus*, *S. typhimurium* and *E. coli* (30). From these studies, it was concluded that probiotic strains are not only used to balance the gut microbiota but they also can potentially modulate the function of immune system.

Macrophage or dendritic cells (DCs) engulf probiotic bacteria and trigger the immune response. DCs present in the intestinal lamina properties has been founded to extend their dendrites between intestinal epithelial cell (IECS) which enhance them to release a different type of cytokines, which subsequently modulate the immune function of DCs, T cells and B cells in the gut-associated lymphoid tissue (GALT). Exposed of probiotic strains to the macrophage and DCs led to release a different type of cytokines like IL-12 and IL-10 which is the key way to control the balance the immune response, so IL-12 activates the cellular immunity whereas IL-10 suppresses the inflammatory responses (Figure 1) (31).

4.3.3. Effects of Probiotics on Diarrhea

The application of the probiotics in the cases of diarrhea, particularly the one caused by rotavirus diarrhea has been shown to be successful using *Lactobacillus* GG strain (32, 33). It has also been suggested that the enzyme ß-galactosidase produced by lactobacilli may be useful in preventing diarrhea in individuals with acquired deficiency of this enzyme (34).

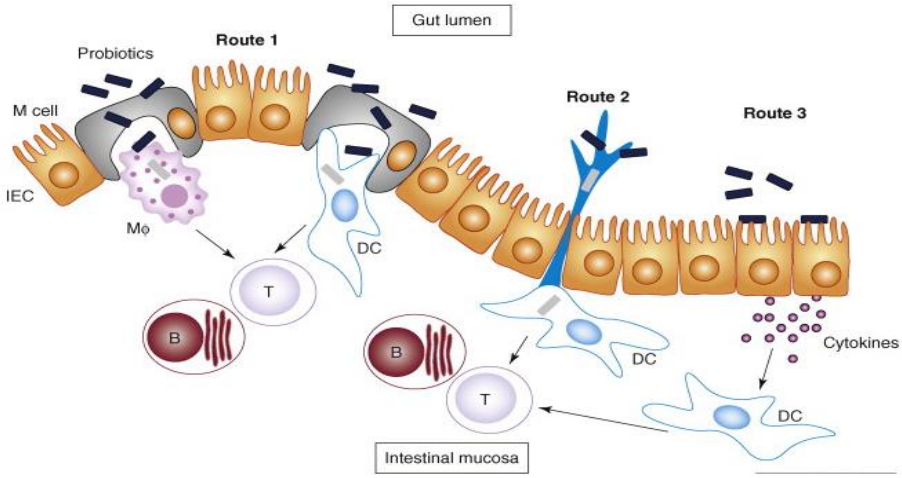

Figure 1. Three hypothetical mechanisms by which probiotic strains can enhance and modulate immune function in the intestine (31).

4.3.4. Effects of Probiotics on Cancer

Probiotics as natural food have been approved as anticancer supplements against different types of cancers. Probiotics perform an anti-carcinogenic action by removing pathogenic agents by competing for nutrients and receptors, releasing of antimicrobial metabolites and inter activating host immune system. Antimicrobial action of metabolites produced by probiotics confer protection against cancer, reducing mutagenicity, diminishing the genotoxicity of dietary carcinogens by mitigating xenobiotic metabolism, regulating apoptosis and suppressing tumor proliferation. Also, probiotics change food preference, influencing the physiology and mental health-conferring the reduced risk of carcinogenesis (35).

Cell-free supernatants (CFS) from *L. casi* and *L. rhamnosus*, which have been observed to prevent colon cancer, cell invasion suggesting that probiotic CFS has anti-metastatic bioactive substances that may participate in cell invasion decrease *in vitro* (36). Eating a large number of dairy products, particularly fermented dairy food such as milk mixed with *Lactobacillus* or *Bifidobacterium* and yogurt may lower the occurrence of colon cancer (37).

4.3.5. Effects of Probiotics on Cholesterol Reduction

Several investigators confirmed that consumption of probiotics bacteria leads to cholesterol reduction by binding or combining directly with cholesterol into the cell membrane. Thus, the enzyme that hydrolysis bile salt may deconjugate these salts which

are easier to be exerted eventually increase the breakdown of cholesterol (38). The results of a study documented that the cholesterol level reduced to 38% when the *L. reuteri* CRL 1098 fed to the mice during 7 days at the level of 10^4 cells/day. This diet resulted in 40% reductions of triglyceride and led to 20% increase in the percentage of high-density lipoprotein (HDL) to low-density lipoprotein (LDL) without bacterial translocation of the native microflora into the spleen and liver (39).

4.4. Mechanism of Probiotics Action

Although the action mechanism of probiotic microorganisms is not well defined, production of antimicrobial substances like bacteriocins, hydrogen peroxide, and organic acids which act as inhibitory agents against gram-positive and negative bacteria, and block and limit the adhesion sites for the colonization of pathogenic agents (40, 41).

4.5. Selection Criteria for Probiotics

The desirable features that potential probiotics are expected to have are listed in Table 1. The safety of the probiotic microorganisms is the most important features. Many approaches have been taken for the evaluation of probiotic strains involving the pharmacokinetic activities of the probiotic strain and its interactions with the host (42).

Table 1. Selection Criteria for Probiotics (41)

Probiotics strains properties	Remarks
Originated from a human for human usage	Although *S. boluardii* is not recovered from human the important of these criteria are for selected species dependent health effects
Acid and bile salt resistance	Important for the oral consumption even may be not used for the other applications, for survival through the intestine, maintaining antiadhesive and metabolic activity.
Adhesion to the intestinal mucosa	Important to support the immune system, competition with pathogens, maintaining anti-adhesive and metabolic activity and prevention of the pathogenic agent to adhesion and colonization
Clinically validated and documented health effects	Minimum effective dosage has to be known for each particular strain and in different products.
Good technological properties	Survival in products if the viable organisms are required phage resistance, stability, culturable in large scale and have no negative effect on flavor products.

4.5.1. Acid and Bile Salt Resistance

Probiotic strains must be resistance to the adverse effects of bile salts. Bile salts are produced from cholesterol in liver and released from gall balder into the duodenum. These salts have antimicrobial actions by disruption of bacterial cell membrane and damaging the DNA in liver (43). Therefore, intestinal microflora that inhibit the intestinal system must have intrinsic tolerance to overcome the lethal effect of bile acids. It has been well documented that *Lactobacillus* and *Bifidobacterium* have a different protein adapted to the efflux of bile salts or proton, also has the ability to modify carbohydrate metabolism or hinder protein misfolding to overcome the harm of bile salts (44).

In a study, 5 out 47 selected *Lactobacillus* strains as potential probiotic bacteria including *L. rhamnosus* 19070, *L. reuteri* DSM 12246, *L. rhamnosus* LGG, *L. delbrucekii* subsp. *lactis* CHCC 2329, and *L. casei* subsp. *lactis* CHCC 3137 showed tolerance under pH 2.5 as well as under 0.3 of oxgall bile salt (45). It was documented that bifidobacteria from human fecal samples were screened for their potential probiotic properties. The recovered strains were tested under pH 2.0, 3.0 and 7.0 and at the level of 0.1 % and 0.05 % of oxgall. The bifidobacteria designated as HJ 30 and SI 31 showed higher tolerance for survival under 1 % and 0.5 % of oxgall for 12 h duration (46).

4.5.2. Antimicrobial Activity

Antimicrobial properties of probiotic bacteria are also important to hinder the entrance of threatening infectious agents to the human body. Antimicrobial properties of probiotic strains are acted depending on some inhibitory materials such as different type of organic acid which includes lactic acid and propionic acid, also by production of CO_2, H_2O_2 and by releasing of antimicrobial peptides such as bacteriocins (41).

Many researches have focused on the role of bacteriocins. The production of bacteriocins vary between strains. In general, bacteriocins exhibit inhibitory effects against closely related bacterial species like *Lactobacillus*, spore forming *Bacillus* and *Clostridium* (40).

In two separate studies, antimicrobial activities of lactobacilli, bifidobacterial and propionic acid bacteria have been shown against *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *S. marcescens* and *C. albicans* that lead to food toxicoinfection and toxemia isolated from dairy and milk products (47, 48). In another work, bacteriocins producing strains of *L.*

fermentum, *Pediococcus* spp., *L. mesenteriodes subsp. mesenteriodes* and *Lactococcus* strains showed antibacterial actions against *E. faecalis* CIP 103907, *B. cerus* LMG 13569, *S. aureus* ATCC 25293, and *E. coli* CIP 105182 (49). Six out 100 *Lactobacillus* strain from vaginal samples displayed inhibitory activity against *G. vaginalis*, *P. aeruginosa*, *P. vulgaris*, *E. coli*, *E. cloacea*, *S. milleri* (50). In another study, *L. reuteri*, *L. plantarum*, *L. mucosa*, *L. rossia* isolated from pig feces added to the feed of the animals showed inhibitory activity against different groups of pathogens including *S. typhimurium* ATCC 27164, *E. coli*, *C. perfringens* 22G, *S. aureus* ATCC 25923, *B. megaterium* F6, *L. innocua* DSM 20649, and *B. hyodysenterias* ATCC 27164 while no inhibitory activity against *B. hydrodysenteria* ATCC 27164 was observed (51).

Olivares and colleagues (52) have also reported the antimicrobial activity of lactobacilli isolated from human breast milk. In their report, *L. salivary* CECT 5713 showed high antibacterial activity against *S. choleraesuis* CECT 4155 in a mouse infection model. It was suggested that (53, 54) some probiotic strains were reported to prevent the adhesion of the pathogens by competitive exclusion (Figure 2).

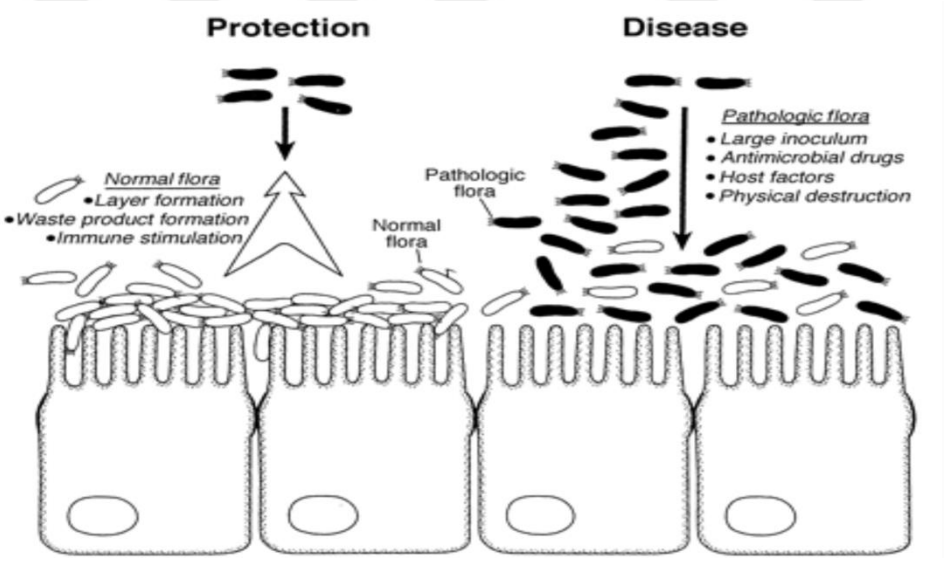

Figure 2. Compete mechanism of normal flora (probiotics) against the intestinal pathogen (54).

4.6. Safety of Probiotics

Safety of probiotic microorganism involved several specifications, one of which is its origin. The probiotic strain which is used for humans must have originated from the

human. In addition, they must have isolated from the healthy intact human digestive system, they must be non-pathogenic, they have no recorded data regarding any type of diseases such as those related to infective endocarditis or those associated with gastrointestinal problems, metabolically they never deconjugate bile salt, and they should not transfer drug resistance genes (42).

4.7. *Lactobacillus*

The genus of *Lactobacillus* bacteria belongs to the wide group of the LAB, which is widely known group with the ability to produce large amount of lactic acid upon utilization of carbohydrate. They phylogenetically belong to the phylum Firmicutes (55). *Lactobacillus* are gram-positive, non-spore-forming, morphologically rod or coccobacilli with G+C content below 50% in molecular weight (56). At the present, about 80 species of *Lactobacillus* are identified (57). They are strictly fermentative microorganism they belong to either homofermentative group which produce mostly lactic acid as end product utilizing glucose as a carbon source, or heterofermentative group which produce lactic acid, CO_2, ethanol, and /or acetic acid (Figure 3).

Lactobacilli are aerotolerant or anaerobic, also able to grow under high acid conditions as acidophilic or aciduric. Lactobacilli are fastidious microorganism that require many nutritional materials for growth like different sugars, amino acids, fatty acid esters, salts, nucleic acids, and vitamins. Lactobacilli are present in different niches which is rich in carbohydrates like plants fermented foods and within the animals (58).

The probiotic species of *Lactobacillus* mostly species like *L. acidophilus*, *L. fermentum*, *L. paracasi*, *L. brevis*, *L. gasseri*, *L. plantarum*, *L. bulgaricus*, *L. helveticus*, *L. casi*, *L. reuteri*, *L. jensenii*, *L. rhamnosus, L. crispatus* (59). Lactobacilli are able to grow between 15-40 ^{o}C temperature, but they are mostly mesophilic, some species are psychrotrophic and thermophilic. Some species of *Lactobacillus* can grow under high concentration of bile salt, high osmotic pressure, and low water activity. Resistance to a high level of acidity remains the most general properties of lactobacilli, they are able to survive under the pH 4.4, the optimum growth is at pH 5.5-6.5 (60).

4.8. Inhibitory Materials Produced by *Lactobacillus*

4.8.1. Bacteriocins

Bacteriocins are protein in nature and possess antimicrobial properties. They possess bactericidal or bacteriostatic activity against both gram-positive and negative bacteria that are closely related or unrelated. Bacteriocins are mostly small, heat-stable, cationic, generally heterogeneous group of antimicrobial peptides or proteins containing 20 to 60 amino acids (61), and they exhibit combined effects with vancomycin for controlling the biofilm formation by *L. monocytogenes* (62). Some *Lactobacillus* bacteriocins can inhibit the growth of different microorganism such as gram-positive and spoilage bacteria and yeasts (63).

4.8.2. Organic Acids

The end product of lactobacilli fermentation during glucose utilization is the lactic acid, while less amount of both acetic acid and succinic acid are produced. Production of lactic acid leads to a decrease in pH of the environment which in turn inhibit the growth of infectious agents. The preservative ability of probiotic bacteria in the food is due to the releasing of some antimicrobial compounds such as organic acid and bacteriocins (64). In addition, lactobacilli are able to produce formic, caproic, propionic, butyric and valeric acid that can exhibit antimicrobial activities. Acids exert their inhibitory activity disturbing the stability of cell membrane potential, preventing active transport, decreasing intracellular pH and hinder the functionality of metabolism process, also have various inhibitory mechanism against the gram-positive and negative group in addition to the yeast and molds.

Lactobacilli are predominant inhabitants of vaginal flora that can be inhibits the multiplication of pathogenic bacteria by utilizing the glycogen to produce lactic acid which lowers the pH of the environment (65). Organic acid production also limits the multiplication of pathogenic agents within the foods. The organic acids are also used as flavoring agents and the acceptability of food products depends largely on the flavor profiles (66). The inhibitory action of lactic acid is due to solubility of the undissociated of lactic acid within cytoplasmic membrane and the insolubility of the undissociated lactic acid which lead to the acidification of cytoplasm and disturb proton motive forces which in turn effect the transmembrane pH content and diminishes the amount of energy needs for growth of cells (67).

4.8.3. Hydrogen peroxide Production

Hydrogen peroxide (H_2O_2) is produced by lactic acid bacteria under aerobic growth conditions. Since LAB are unable to produce catalase or peroxidase, they produce H_2O_2 to the surrounding environment to keep themselves from the inhibitory actions of this oxidizing agent (68). H_2O_2 producing lactobacilli are essential to prevent pathogen bacterial colonization in urinary tract and vagina to prevent biofilm formation and limit the growth of pathogens (69-71).

4.8.4. Biosurfactants

Biosurfactants are unique amphiphilic secondary metabolites, produced by microorganisms from different basic materials. Biosurfactants have the ability to reduce the surface tension between immiscible media (72). Different types of biosurfactant exhibit different physiological functions such as stimulating the solubility of hydrophobic/water-insoluble compounds, binding to heavy metal, rolling in the pathogenicity of-of bacteria, aid in cell adhesion and aggregation, role in quorum sensing mechanism and biofilm formation (73).

Several biosurfactants observes different antagonistic activity such as antifungal and antiviral activities, biosurfactant has the ability to change the hydrophobicity of surface which in turn inactivate the adhesive mechanism of pathogenic agents to the infection site (74). *L. rhamnosus* and *L. jensenii* exhibited anti biofilim and anti-adhesive activity against most clinical bacterial strains, especially multi drug resistance strains such as *A. bumanii, E. coli* and *S. aureus* (75).

4.8.5. Nanoparticles

Nanomaterials, natural incidental or manufactured materials containing particles have a dimension ranging in size from 1-100 nm (76). The fast growth and relative ease of genetic manipulation of bacteria aided in the synthesis of nanoparticles. Microbially mediated synthesis of TiO_2 nanoparticles was carried out using *Lactobacillus* sp. (77). A low cost, green reproducible probiotic bacteria *L. sporogens* isolated from the yogurt has been reported to biosynthesize ZnO (78). The antibacterial action of TiO2 nanoparticles against *E. coli* demonstrated that they could be used in different fields and medical applications (79).

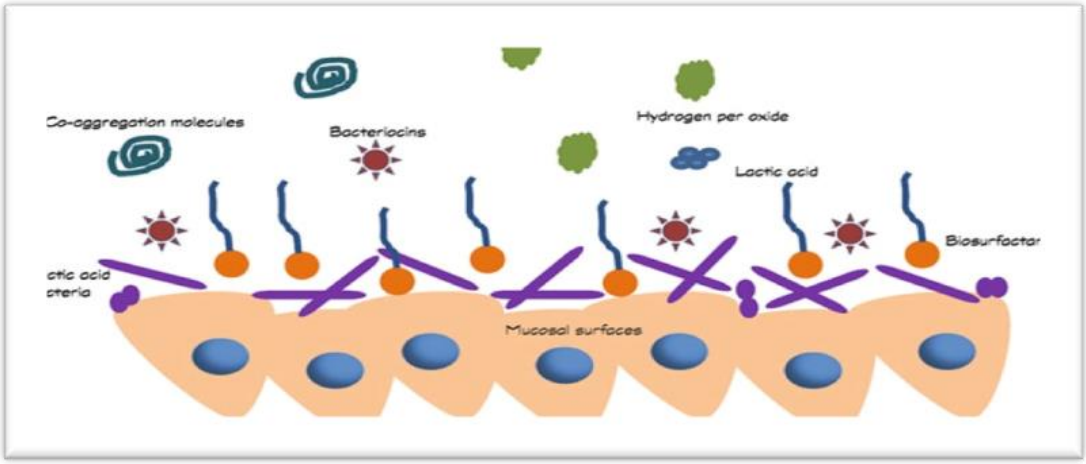

Fıgure 3. Production of various compounds by LAB on mucosal membrane (111).

5. MATERIALS AND METHODS

5.1. Materials

5.1.1. Chemicals

Chemicals, reagents and enzyme used in this study include agarose, EDTA (Quantum, Hollanda), glycerole, hydrochloric asit (HCl), soduim chloride (NaCl) (Riedel de Haen, Germany), Dimethyl sulfoxide (DMSO), potassium chloride (KCl), sodium acetate ($C_2H_3NaO_2$), sodium dodecyl sulphate (SDS), mitomycin C (Sigma-Aldrich, USA), methanol (Chroma Norm, France), horse serum, phosphate buffer (PBS, Dulbecco's A) (Oxoid, UK), ammonium sulphate [(NH4)2SO4], disodium hydrogen phosphate (Na2HPO4), L-arginine, magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), N, N, N", N"-tetra-methyl-p-phenylalanine diamin dihydrochloride, dipotassium hydrogen phosphate (K_2HPO_4), 30% hydrogen peroxide (H_2O_2), isomayl alcohol, pancreatic peptone, potassium dihiydrogen phosphate (KH_2PO_4), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), yeast extract, sucrose, (Merck, Germany), protease peptone No:3 (Becton Dickinson, USA), chloroform (Roche, Germany), lysozyme (Apply Chem, Germany), RNase A (Biomatik, Canada), amoxicillin, azithromycin, chloramphenicol, erythromycin, gentamycin, ciprofloxacin, pencillin, streptomycin, teicoplanin, vancomycin (Bioanalyse, Turkey). Primers; 27F:5'-AGAGTTTGGATCMTGGTCAG-3', 1492R: 5'-GGTTACCTTGTTACGACTT-3' (Macrogen, South Korea), PCR master mix (5x HOT FIREPol® Blend Master Mix), 100 bp DNA marker (Solis BioDyne, Estonya), and Big Dye ® (Applied Biosystems, USA) used throughout the study.

5.1.2. Culture Media

Brain heart infusion broth (BHIB), Luria Bertani (LB) broth (Lab M, UK), 5% blood sheep agar (OR-BAK, Turkey), Demand Rogosa agar (MRSA) and broth (Lab M, UK) were used.

5.1.3. Bacterial Strains

Gram-negative and gram-positive bacteria and fungi (ATCC type strains) were obtained from Medical Microbiology Department of Karadeniz Technical University. Strains used include; *C. albicans* ATCC 10231, *P. mirabilis* ATCC 13315, *S. typhi* ATCC 14028, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 13883, *E. coli*, ATCC 29212, *S. pyogenes* ATCC 12344 and *E. faecalis* ATCC 29212, which were

used for evaluating the antimicrobial activity of isolated lactobacilli. *L. acidophilus* ATCC 4356, *L. fermentum* ATCC 14931 and *L. reuteri* ATCC 23272 were used for the evaluation of bacteriopahge and/or bacteriocin production.

5.2 Methods

5.2.1. Preparation of Solutions and Reagents

The culture media and solutions were sterilized by autoclaving at 121^{o}C for 15 min or by filtration with 0.22 µm and 0.22 µm pore size filters.

5.2.2. Catalase Reagent

This reagent was prepared by mixing equal amount of 6% H_2O_2 with distilled water and stored in a brown bottle at 4^{o}C refrigerator (80).

5.2.3. Oxidase Reagent

Oxidase reagent was prepared by dissolving 1 g of N-N-N-N-tetraethyl-P-phenyldiamine hydrochloride in 100 mL of distilled water. This reagent was prepared fresh daily and stored in a dark bottle in a refrigerator.

5.2.2.4. Mitomycin C

Mitomycin C was used for bacteriophage (prophage) induction from bacterial genomes. Mitomycin C was dissolved in distilled water at a final concentration of 1 mg mL^{-1} and stored at -20°C until used.

5.2.5. Preparation of the Culture Media

All microbiological growth media were prepared according to the instructions of the manufacturers. The desired pH of each media was checked and if necessary and adjusted before autoclaving. Prepared agar plates were stored at 4 °C and the liquid media were stored at room temperature unless stated otherwise.

5.2.6. Preparation of Carbohydrate Solutions

Solutions of arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melebiose, melezitos, raffinose, rhamnose, salicine, sorbitol, sucrose and xylose were prepared by dissolving 1 g (10%) from each type in 10 ml of distilled water. The mixture was sterilizing by filtration through 0.22 µl filter and stored in a

refrigerator. Each of the sugar solution was mixed with the bromocresol purple (BPB) medium to to obtain working solution of 0.5%.

5.3. Samples Collection

About fifty different specimens of fermented products from both homemade and commercial products including different types of cheese, olives, pickles, sausages, turnip juices and yogurts were obtained from randomly selected local markets in the city of Trabzon. Samples were placed in sterile plastic bags and/or bottles and transported under cooled conditions to the laboratory for culturing.

5.4. Isolation of *Lactobacillus* Strains

From each liquid or semi-solid sample approximately 50 µl was taken either with a loop or pipette and streaked on MRSA agar media. From solid samples such as cheese, olives, pickles and sausages approximately 5 g were homogenized in 45 ml of phosphate buffer (PBS) and 50 µl from homogenized mixture were streaked on agar media as described above. All cultures were incubated at 37°C for 48 h under 5% CO_2 conditions. Colonies with different morphologies were picked and primary identification of the strains were made based on Gram staining, cell morphology, catalase and oxidase test results. The isolates that observed as gram-positive bacilli with white yellow, large, smooth, round colony characteristics, catalase and oxidase negative results were selected as presumptive lactobacilli. The selected lactobacilli strains were further sub-cultered for purification and stored in 20% glycerol at −80 °C.

5.5. Identification of *Lactobacillus* Strains

5.5.1. Colony Morphology

The appearance of colonies on the MRS media was examined with regarded to shape, colour, size, opacity, and margin as described previously (80).

5.5.2. Microscopic Examination

All the bacterial isolates were examined microscopically for Gram stain reaction, shape, and arrangement as previously reported (80).

5.5.3. Biochemical Tests

5.5.3.1. Catalase Test

An 18 h old bacterial colony was transferred onto a clean glass slide with sterile wooden sticks and mixed with a small drop of 3% H_2O_2. Absence of gas bubble within 20-30 seconds indicated the absence of catalase enzyme in the tested strain.

5.5.3.2. Oxidase Test

A filter paper was saturated with oxidase reagent then an18 h old bacterial colony was transferred to the filter paper and rubbed onto the reagent with a sterile wooden stick. The purple color appearance in 30 seconds indicated a positive result.

5.6. Physiological and Biochemical Tests

5.6.1. Carbohydrates Utilization

Suspected *Lactobacillus* strains were further identified by their carbohydrate fermentation ability of 17 sugars by the method described previously (81) with some modifications. Briefly, *Lactobacillus* cells suspension and sugar solutions were prepared separately. About three discrete colonies from fresh culture plates were transferred to an eppendorf tube containing 500 µl of BPB media without any sugar. The mixture then subjected to washing by centrifugation at 10,000 xg for 1 min. The supernatant was decanted and the washing repeated twice. The pellet was then suspended in 500 µl of BPB media and 50 µl from this suspension was transferred to ferntation tubes containing 1 ml of BPB media with one of the 17 sugars at 0.5% concentration. The cultures were incubated at 37^{o}C for up to 24 h and monitored for color changes to yellow which is the indication of positive result. Glucose was used as positive control.

5.6.2. Gas Production from Glucose

The purpose of this test was to classify homonfermentative and heterofermentative bacteria during fermentation of glucose. Modified MRS broth without ammonium citrate was prepared and distributed in 5 mL glass tubes with inverted durham tube aiming to detect the production of CO_2 during consumption of glucose. The tube was inoculated with 18 h culture at 1% final concentration and incubated at 37^{o}C for 5 days. Appearing of gas inside the durham tube was considered as an indication of production of CO_2 as previously reported (82).

5.6.3. NaCl Tolerance

To determine the growth rate of lactobacilli with different concentrations of MRS broth media containing NaCl ranging from 1 to 10% were distributed in test tubes. Each tube was inoculated with overnight culture of *Lactobacillus* and incubated for 24 h. The salt resistance was determined by observing the turbidity of the culture as described previously (83).

5.7. Assessment of the Probiotic Properties of *Lactobacillus* Strains

All *Lactobacillus* isolates obtained from 50 different dairy products were subjected to different tests to evaluate their ability to act as probiotic bacteria. First, major selection properties were focused on two properties such as ability to grow under different pH and tolerance to high bile salt concentration. Second, the strains that exhibited resistance to both acidity and bile salt concentration would only be selected for other properties like resistance to pepsin and pancreatic enzymes, sensitivity to antibiotic, absence of haemolytic activity, adherence ability to mammalian cells (HT-29 cells) and inhibitory compounds that they produce.

5.7.1. Acid Tolerance

Acidity tolerance test was employed by the method described before (84). In brief, an optical density of each *Lactobacillus* growth was adjusted to 0.6 on MRS broth at OD 600 nm. Then, 2% of culture was transferde to the five separated tubes containing MRS broth with pH adjusted to 2, 3, 4, 5 and 6.5 with 4 M HCl. All cultures were incubated for 24 h and growth rate was determined by measuring the optical density.

5.7.2. Bile Salt Tolerance

The ability of selected *Lactobacillus* to grow under high bile salt concentrations was evaluated by the procedure suggested (85) with slight modifications. MRS broth with varying concentrations (0.3%, 0.15%, 0.06%) of bile oxgall was prepared and autoclaved at 121°C. Then, 2% overnight cultures of *Lactobacillus* were inoculated into each of the three MRS broth with different concentrations of bile. All cultures were incubated for 3 h and 6 h under microaerophilic conditions. The growth rate was assessed by taking the OD readings at the time of incubation (0 h) for each concentration and after 3 h and 6 h of incubation.

5.7.3. Hemolytic Properties

The haemolytic properties of *Lactobacillus* strains were determined by following the method suggested previously with some modifications (86). A loopful culture of *Lactobacillus* strains grown overnight in MRS broth was spread over Columbia blood agar medium supplemented with 5% sheep blood. The cultures were for 16 h under microaerohipic conditions and hemolyic properties were assessed using *E. coli* ATCC 29212, *S. aureus* ATCC 25923 and *E. faecium* ATCC 6057 as controls.

5.7.4. Resistance to Antibiotics

The antibiotic susceptibility was evaluated by using disc diffusion method (87). Briefly, suspension of each *Lactobacillus* strains was adjusted to a McFarland standard of 0.5, and a cotton swab was soaked in adjusted suspension and spreaded evenly on MRSA agar. The selected antibiotic discs including penicillin (10 U), amoxicillin (30 µg), vancomycin (30 µg), teicoplanin (30 µg), azithromycin (15 µg), chloramphenicol (30 µg), streptomycin (10 µg), gentamycin (10 µg) and erythromycin (15 µg), and ciprofloxacin (5 µg) were evenly placed on agar surface and cultures incubated for 24 h. The inhibition zone diameters (IZD) were measured and evaluated.

5.7.5. Pepsin and Pancreatin Resistance

Briefly, 1 mL culture grown in MRS broth was transferred to eppendorf tube and subjected to centrifugation at 10,000 x g for 5 min. Supernatant was decanted and pellet was washed with PBS twice (pH 7.2), and the pellet was resuspended in 3 mg mL^{-1} of freshly prepared pepsin (pH 2.0) and 1 mg mL^{-1} pankreatin, respectively. Cell viability was determined on MRS agar by plating 100 µl pepsin and pancreatin treated cells after 3 h and 4 h of incubation at 37˚C, respectively. Cell suspensions without enzymes at zero time (0 h) was used as control. The number of pepsin and pancreatin resistance cells were calculated as CFU/mL at the end of 24 h incubation period (86).

5.7.6. Adhesion Capacity

Human colon adenocarcinoma cell line HT-29 was used for evaluation of adhesion capacity of selected lactobacilli using the method described elsewhere (88) with some modifications. Briefly, HT-29 cells were activated in Dulbecco's Modified Eagle Medium (DEMEM) supplemented with 10% fetal calf serum (FBS) and antibiotics (100 U mL^{-1} pencillin and 100 µg mL^{-1} streptomycin) at 37 °C in humidified atmosphere with 5% CO_2.

HT-29 cell line was seeded onto a 24-well tissue culture plate at a concentration of $5x10^5$ cells mL^{-1}. After 24 h of incubation, the medium was replaced with unsupplemented medium and a monolayer of HT-29 was obtained. *Lactobacillus* strains were prepared at a concentration of $5x10^7$ cells mL^{-1} and finally resuspended in DEMEM medium and 1 mL of each *Lactobacillus* suspension was added to wells of the culture plate. Adherence capacity was evaluated after 2 hr of incubation. After 2 h of incubation the bacteria which had not attached to the HT-29 cells were removed by washing three times with PBS containing Ca^{2+} and Mg^{2+} ions (pH 7.4). PBS was decanted by pipetting. In order to separate the adherent bacteria from the HT-29 cells, the HT-29 cells were lysed using 0.1% (v/v) Triton-X100 and the number of adherent bacteria were counted by plating of suitable serial dilutions on MRS agar medium. The adhesion index of each *Lactobacillus* bacteria was calculated as the number of adherent bacterial cells per 100 HT-29 cells.

5.7.6.1. Adhesion Score

Adhesion score assay was evaluated by following the procedure suggested elsewhere (89) with some modification. The cell suspension of HT-29 cell line was prepared at a concentration of $1x10^5$ in 4 mL in DMEM medium. The mixture was then distributed to each well of the six-well tissue plate. The plate was incubated for three days until confluency reached to 80 per cent. During this incubation period the media was changed every day and before one hour of test with unsupplemented media. *Lactobacillus* cell density adjusted to $1x10^8$ CFU were suspended in 1 mL DEMEM medium without FCS and antibiotics and transferred to each selected well. The plates were incubated in a CO_2 incubator for 2 h. The monolayer cells were washed five times with sterile PBS (pH 7.4), the cells were fixed with methanol and stained with 0.5% crystal violet. The adhesion score was measured by enumerating the adhered bacteria per 20 different microscopic fields. The adhesion scores were grouped into non-adhesive (≤ 40 bacteria), adhesive (41-100 bacteria) and strongly adhesive (>100 bacteria).

5.8. Characterization of Antimicrobial Substances Produced by *Lactobacillus*

The well diffusion method described elsewhere (90) was used with slight modification to determine the type of antimicrobial substances produced by *Lactobacillus* strains against some pathogens. *Lactobacillus* strains were subcultured in MRS broth while the selected pathogens were grown in suitable media and incubated at 37°C overnight. Cultures of each *Lactobacillus* strains were subjected to centrifugation at 4000 xg for 10

min at 4°C and the supernatant was separated. The supernatant was aliquoted into three tubes and the first tube was treated with 1 mg mL^{-1} trypsin for determining of bacteriocine production. The second tube was treated with 0.5 mg mL^{-1} catalase for determining hydrogen peroxide production, and the third tube was adjusted to pH 6.5 ± 0.1 with 1 NaOH, and the fourth tube was used as positive control (non-treated). The treated supernatants were sterilized by filtration. Wells were punctured into the Muller Hinton (MHA) agar plates which were previously seaded with each of pathogenic bacterial culture adjusted to 0.5 McFarland. To each well 100 µl treated supernatant was transferred and plates were kept in the refrigerator for 3 h to allow diffusion of the supernatant into the agar. Then all the plates were incubated at 37 °C for 48 h. The inhibition zone of each treated well was measured. The type of antimicrobial substance responsible for pathogen inhibition was determined based on the presence/ absence of inhibition zones.

5.9. Evaluation of *Lactobacillus* Strains for Bacteriophage Carriage and Bacteriocin Production

To evaluate the presence of prophage in the chromosomes of the dairy *Lactobacillus* isolates mitomycin C induction method was utilized as described previously (91). Production of the bacteriocins upon mitomycin C induction was also employed. Briefly, 100 µl of *Lactobacillus* cultures transferred into test tubes containing 10 mL of two type of media supplemented with calcium carbonate adjusted to different pH. The first medium used was MRS-C with pH 6.2 and 5.5, the other medium was Rogosa (SLA-C) with pH 5.5. After 3 h of incubation, the broth of each *Lactobacillus* was separated into two test tubes. The first one contained mitomycin C at a final concentration of 0.2 mg mL^{-1} while the other was used as control (without mitomycin). *L. acidophilus* ATCC 4356, L. *fermentum*, ATCC 14931 and *L. returi* ATCC 23272 were used as indicator strains for both phages and bacteriocins. The results were assessed by using agar spot assay (91).

5.9.1. Agar Spot Assay

The result of induction of *Lactobacillus* phages or bacteriocins was indicated by a clear lysis of the turbid culture 5 to 24 h after the addition of mitomycin C. The lysates were subjected to centrifugation and filtered to remove the remnant bacteria or unlysed bacterial cells. Three µl of each lysate was transferred onto the soft MRS agar (0.6 %) seeded with the indicator strains and incubated at 37 °C for 24 h. Presence of phage infection or bacteriocin were evaluated by the presence of clear and or turbit lysis zones in

the soft agar layer (91). The unused lysates were treated with a drop of chloroform and kept in 4°C for later use.

5.10. Characterization of Biosurfactant Production

5.10.1. Isolation of Biosurfactants

For production of biosurfactant from selected *Lactobacillus* strains, 2 mL from each overnight culture of bacteria were mixed with 100 mL of MRS broth in erlenmeyer flask and incubated at 37°C for 72 h at the stationary state as described previously (92). After 72 h cell-bound biosurfactant were isolated. For isolation of the cell-bound biosurfactants, the method described elsewhere was used (93) with some modification. In brief, cell biomass of each bacterial strain was collected by centrifugation at 10,000 ×g, 5 min at 10°C, then washed twice with distilled water, and suspended in 10 mL of PBS (pH 7,0). The bacterial suspensions were incubated at room temperature for up to 4 h with gentle stirring for releasing the biosurfactant. Bacteria were removed by centrifugation and the cell-free supernatant was regarded as crude biosurfactant.

5.10.2. Evaluation of Biosurfactant Production

The ability of *Lactobacillus* bacteria to produce biosurfactant was assessed using following three methods.

5.10.2.1. Oil Spreading Test

This test was done by using three type of oil as substrate for determination of cell bound biosurfactant as previously described (93). Fifty milliter of distilled water was poured in the petri dish, then the petri dish was overlaid with 20 µL of kerosene or vegetable oils (sunflower and olive oil) separately. Immediately, 10 µL of crude biosurfactant dissolved in PBS was loaded on the surface of oil and the diameters of clear zones were measured. Triton X- 100 was used as the positive control while PBS served as the negative control. This test was repeated three times.

5.10.2.2. Drop Collapse Test

Drop collapse test was performed as previously reported (94) with slight modification. A 2 µl of kerosene was loaded to the wells of 96-well microplate lids and allowed to equilibrate for 24 h duration. Then 5 µL from the crude biosurfactant was transferred to the oil coated regions and the drop size stability was observed with the aid of

a magnifying glass within 1 to 3 min. A result was scored positive for biosurfactant production when the drop diameter was at least 1 mm larger than that produced by de-ionized water (negative control). Triton X-100 (a chemical surfactant) solution prepared at 1 mg mL^{-1} concentration was used as a positive control. The experiments were repeated three times.

5.10.2.3. Emulsification Index

To measure the Emulsification Index (EI) for the crude biosurfactant produced from the selected *Lactobacillus* strains, the protocol used was described elsewhere (92). Briefly, 1 mL of crude biosurfactant mixture was transferred to the graduated glass test tubes. Then three mL distilled water was transferred to the same tube. One mL from each type of oil kerosene, sunflower and olive oil was transferred to each tube separately. The tubes were shaken vigorously for 2 min and the mixture of each tube was left at room temperature for 24 h. At the end, the height of the formed emulsion was measured, and emulsification index was calculated. Water was used as negative control while Tween-1000 (1%) served as the positive control. All experiments were repeated three times. The emulsification index (EI) was evaluated using the following formula:

EI 24 = Height of emulsion layer / Total height x100

Where, EI 24 = emulsification index after 24 h incubation

5.10.2.4. Antimicrobial Assessment of Biosurfactant Production

The inhibitory activity of cell-bounded crude biosurfactant was evaluated against several types of pathogens by using microdilution method in 96 flat-well plates. Briefly, 125 µl of sterile double strength medium (TSB) were transferred into the first column of the 96-well plate, and 125 µl of sterile single strength medium were loaded in the other wells. Then, 125 µl of biosurfactant dissolved in PBS (50 mg mL^{-1}) were transferred to the first column of the plate and mixed by using multichannel pipet. This resulted in a biosurfactant concentration of 25 mg mL^{-1}; then serially, 125 µl were transferred to the subsequent wells, discarding 125 µl of the mixture in the tenth column, so that the final volume for each well were 125 µl. The two-fold serial dilution of the biosurfactant in the first 10 wells resulted in 25 to 0.048 mg mL^{-1} concentration. The columns 11 and 12 did not contain biosurfactant and served as negative and growth controls, respectively. All the wells except the 11^{th} column was inoculated with 2.5 µl from each pathogenic bacterium

prepared at a final concentration of 1 x10^8 CFU mL^{-1}. The plates were kept at 37°C for 48 h. The growth percentage of each bacterium under different biosurfactant concentration was calculated by measuring the optical density of each well using following formula;

$$\% \text{ Growth} = OD_C / OD_0$$

Where OD_C referred to the optical density of the wells with a crude biosurfactant, and OD_0 represented the optical density value of the control well without biosurfactant. The test was repeated three times (95).

5.11. Molecular Identification of Lactobacilli

5.11.1. Genomic DNA Isolation

For extraction of *Lactobacillus* chromosomal DNA, phenol chloroform isoamyl alcohol method was followed (96). Briefly, 1.5 to 3 mL overnight culture was transferred to eppendorf tubes and centrifuged at 10,000 xg for 3 min. The pellets were resuspended in 200 µL of lysis buffer (40 mM Tris-Acetate pH 7.8, 20 mM Na-acetate, 1 mM EDTA, 1% SDS). The mixture was vortexed for 2 min. After that, 10 µL (10 mg mL^{-1}) lysozyme was added and incubated at 37°C for 30 min. Then, 10 µL of 10 mg mL^{-1} RNase I was added to the mixture and incubated at 56°C for 30 min or until complete lysis occurred. After complete lysis, 70 µL 5 M NaCl was added to the tube and mixed well. The mixtures were then centrifuged at 10,000 xg for 10 min. The clear lysate of mixture was transferred into a sterile eppendorf tube and equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inverting gently at least 50 times. The mixture was again centrifuged at 10,000 xg for 10 min and the lysate was again transferred to another eppendorf tube. Then, two volumes of 100% cold ethanol were added to the tube. The mixture was incubated at -20°C for 30 min. For precipitation of DNA, the mixture was centrifuged at 10,000 xg for 5 min. The pellet was washed with 70% ethanol twice by adding 100 µL ethanol and centrifugation at 10,000 xg for 1 min. Pellet was dried at 50°C, and the DNA was resuspended in 50-100 µL TE solution. The DNA was stored at -20 °C until used.

5.11.2. Amplification of the 16S rDNA Region of *Lactobacillus*

Two universal primers, 27F and 14925R, for 16S rDNA were used for apmplification. The PCR amplification was undertaken in 25 µL reaction volume containing the following compositions: 5 µL PCR buffer (Promega 5x), 0.5 µL dNTP (2.5 µM), 0.6 µL of each primer (10 pmol μL^{-1}), 2.5 µL of $MgCl_2$ (25 mM), 0.15 µL (5U μL^{-1})

Taq DNA polymerase (Promega), 2 µL of *Lactobacillus* DNA, and 13.65 µL of sterilized distilled water. The PCR conditions was adjusted as follows; initial denaturation at 94^{o}C for 5 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72 °C for 1 min, and final extension at 72 °C for 10 min.

5.11.3. Analysis of PCR Products by Agarose Gel

The amplicon of each *Lactobacillus* 16S rDNA gene fragment was analyzed by gel electrophoresis. The agarose gel was prepared at 1.2% concentration and stained with ethidium bromide. Then 5 µl from each amplicon was loaded onto the selected well. For detecting the right size of the *Lactobacillus* amplicon, a DNA marker was loaded in the first well of the gel. The PCR products were electrophoresed at 70 volts for 45 min and the gel was visulaized under UV illuminator.

5.11.4. Purification of the PCR Products

The PCR products were purified by using MACHEREY- NAGEL gel and PCR clean- up kit according to the manufacture's instructions.

5.11.5. Sequencing of 16S rDNA

To perform sequencing of purified PCR amplicons, Applied Biosystem ABI 373x1 DNA Analyzer with BigDye® v3.1 Cycle sequencing kit (Applied Biosystem®, USA) and 27F and 1492R universal primers were used. The sequence reaction was carried out in a total of 10 µL reaction volume consisting of 2 µL of purified PCR amplicon, 1.5 µL of 5x sequencing buffer, 1 µL of BigDye v 3.1; 0.32 µL of each forward/reverse primer (10 pmol/µL) and 5.18 µL of sterile distilled water. After the reagents were mixed, PCR was run with the following PCR conditions; 3 min of initial denaturation at 96^{o}C followed by 25 cycles of denaturation at 96^{o}C for 10 sec, annealing at 50^{o}C for 5 sec, extension at 60^{o}C for 4 min, and a final extension at 4^{o}C. The resulting PCR products were purified by passing through a spin column containing polymerized Sephadex G-25®. The purified product was loaded onto microtiter plate of the DNA sequence Analyzer and the results of the sequencing were analyzed. The similarities between strains were determined by comparing the data with the corresponding 16S rDNA sequences published in GenBank database.

5.12. Identification of *Lactobacillus* Strains by Matrix Assisted Laser Desorption / Inoization, Time of Flight Mass Spectrometry

A portion of a colony of lactobacilli in question was placed onto the sample target and overlaid with matrix. The mass spectra generated were analysed by dedicated software and compared with stored profiles.

5.13. Statistical Analysis

The experimental results were expressed as mean±standard deviation (SD) for analysis performed in triplicates. Statistical analysis of the data was performed by using SPSS software version 22.

6. RESULTS

6.1. Isolation of *Lactobacillus* Species

A total of 50 different fermented products from both homemade and commercial product including different types of cheese, olives, pickles, sausages, turnip juice and yogurt were purchased from different local markets in the city of Trabzon city which selected randomly (Table 2). Based on the culture results, 103 *Lactobacillus* spp. were mostly obtained from the samples of white cheese 55 (53.39 %) while from only 2 (5.8%) pickles and turnip samples *Lactobacillus* were isolated.

Table 2. Number of lactobacilli isolated from different dairy products

Products	Manufacturer	Code	No. of samples	Number of Lactobacilli isolated (%)
Yogurt	Commercial	TCY	15	23 (22.3)
	Homemade	THY	9	12 (11.6)
White	Commercial	TCWC	5	9 (8.7)
cheese	Homemade	THWC	15	46 (44.6)
Olive	Commercial	TCO	2	3 (2.9)
	Homemade	-	-	-
Sausage	Commercial	TCS	2	6 (5.8)
	Homemade	-	-	
Pickles	Commercial	TCP	1	2 (1.94)
	Homemade	-	-	-
Turnip	Commercial	TCT	1	2 (1.94)
	Homemade	-		
Total			**50**	**103**

6.2. Isolation of Lactobacilli from Dairy Products

6.2.1. Cultural Characterizations

Morphological characteristics of colonies were evaluated on MRS agar. All *Lactobacillus* isolates were able to grow on MRS agar presenting white or yellow, large, smooth, round colonies with entire margins (Figure 4).

Figure 4. Morphological features of TLB 504 on MRS agar.

6.2.2. Microscopic Characterization

Microscopic examination showed that the cells of *Lactobacillus* reacted positively with Gram stain appeared as purple in color as short or long rods that arranged singly or in pairs or in short chains under the light microscope as shown in Figure 5.

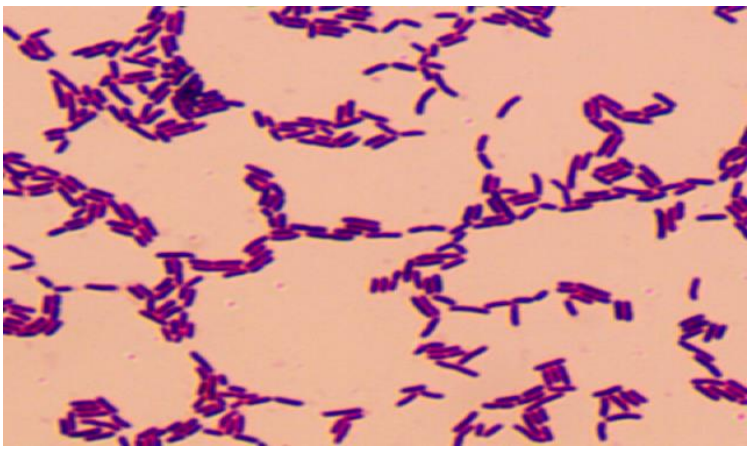

Figure 5. Gram stain of TL 504 (100 X).

6.2.3. Primary Identification of Lactobacilli

This study was aimed for the isolation and characterization of *Lactobacillus* strains from different dairy products for good probiotic properties. About 103 of *Lactobacillus* isolates were primarily identified depending on gram reaction, catalase, oxidase and capacity to ferment 17 different types of sugars. All isolates were gram-positive bacilli or rod-like bacilli with negative catalase and oxidase reactions.

Sugar fermentation result indicated that all 103 *Lactobacillus* isolates were able to consume cellobiose sugar as carbon source while most strains were able to utilize sorbitol and rahminose 51% and 21%, respectively. The fermentation rate of other sugars was moderate to high as demonstrated in Table 3.

Table 3. Sugar fermentation pattern of isolated lactobacilli.

No	Code	ARB	CLB	FRU	GAL	GLU	LAC	MAL	MAN	MNT	MLZ	MLB	RAF	RHM	SAL	SRB	SUC	XYL
1	TLB 500	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
2	TLB 501	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
3	TLB 502	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
4	TLB 503	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
5	TLB 504	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
6	TLB 505	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
7	TLB 506	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
8	TLB 507	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
9	TLB 508	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
10	TLB 509	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
11	TLB 510	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
12	TLB 511	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
13	TLB 512	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-
14	TLB 513	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-
15	TLB 514	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
16	TLB 515	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
17	TLB 516	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
18	TLB 517	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-
19	TLB 518	+	+	+	-	+	+	+	-	+	+	+	+	-	-	+	+	-
20	TLB 519	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-
21	TLB 520	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-
22	TLB 521	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
23	TLB 522	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-

Table 3. (Continued)

No	Code	ARB	CLB	FRU	GAL	GLU	LAC	MAL	MAN	MNT	MLZ	MLB	RAF	RHM	SAL	SRB	SUC	XYL
24	**TLB 523**	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+
25	**TLB 524**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
26	**TLB 525**	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-
27	**TLB 526**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
28	**TLB 527**	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+	+
29	**TLB 528**	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-
30	**TLB 529**	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	+	-
31	**TLB 530**	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	-
32	**TLB 531**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-
33	**TLB 532**	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-
34	**TLB 533**	+	+	+	+	+	+	+	+	+	**WP**	-	+	-	+	+	+	-
35	**TLB 534**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-
36	**TLB 535**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
37	**TLB 536**	+	+	+	-	+	+	+	+	+	-	+	+	-	+	-	+	-
38	**TLB 537**	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+
39	**TLB 538**	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+
40	**TLB 539**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-
41	**TLB 540**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
42	**TLB 541**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
43	**TLB 542**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
44	TLB 543	+	+	+	-	+	+	+	+	+	**WP**	+	+	-	+	-	+	-

Table 3. (Continued)

No	Code	ARB	CLB	FRU	GAL	GLU	LAC	MAL	MAN	MNT	MLZ	MLB	RAF	RHM	SAL	SRB	SUC	XYL
45	**TLB 544**	+	+	+	**WP**	+	+	+	+	+	+	+	+	-	+	-	+	-
46	**TLB 545**	+	+	+	-	+	+	+	+	+	+	+	**WP**	-	+	-	+	+
47	**TLB 546**	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-
48	**TLB 547**	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	-
49	**TLB 548**	-	+	-	-	+	-	+	-	-	+	+	+	-	-	-	+	+
50	**TLB 549**	-	+	-	-	+	-	+	-	+	+	+	+	-	-	-		+
51	**TLB 550**	-	+	-	-	+	-	+	+	-	+	+	+	-	+	-	+	+
52	**TLB 551**	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-
53	**TLB 552**	-	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	+
54	**TLB 553**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-
55	**TLB 554**	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-
56	**TLB 555**	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	+	-
57	**TLB 556**	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+	+
58	**TLB 557**	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-
59	**TLB 558**	+	+	+	-	+	+	+	+	+	+	+	**WP**	-	+	-	+	+
60	**TLB 559**	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+	+
61	**TLB 560**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
62	**TLB 561**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
63	**TLB 562**	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-
64	**TLB 563**	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	+	-
65	**TLB 564**	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-
66	**TLB 565**	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+	+

Table 3. (Continued)

No	Code	ARB	CLB	FRU	GAL	GLU	LAC	MAL	MAN	MNT	MLZ	MLB	RAF	RHM	SAL	SRB	SUC	XYL
67	TLB 566	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-
68	TLB 567	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+	+
69	TLB 568	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	+	-
70	TLB 569	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-
71	TLB 570	+	+	+	-	+	+	+	+	+	+	+	+	-	+		+	-
72	TLB 571	+	+	+	+	+	WP	-	WP	+	+	+	+	-	-	-	+	+
73	TLB 572	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+
74	TLB 573	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	+
75	TLB 574	WP	+	+	+	+	+	+	+	+	+	+	+	-	-	WP	+	+
76	TLB 575	WP	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
77	TLB 576	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
78	TLB 577	WP	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
79	TLB 578	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+
80	TLB 579	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+
81	TLB 580	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
82	TLB 581	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
83	TLB 582	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	WP
84	TLB 583	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-
85	TLB 584	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+
86	TLB 585	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	+	-
87	TLB 586	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-
88	TLB 587	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+
89	TLB 588	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
90	TLB 589	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+

Table 3. (Continued)

No	Code	ARB	CLB	FRU	GAL	GLU	LAC	MAL	MAN	MNT	MLZ	MLB	RAF	RHM	SAL	SRB	SUC	XYL
91	**TLB 590**	**WP**	**+**	**+**	**WP**	**+**	**+**	**+**	**WP**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
92	**TLB 591**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
93	**TLB 592**	**WP**	**+**	**+**	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
94	**TLB 593**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
95	**TLB 594**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
96	**TLB 595**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
97	**TLB 596**	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
98	**TLB 597**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
99	**TLB 598**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
100	**TLB 599**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**+**	**+**	**+**	**WP**	**-**	**-**	**-**	**+**	**+**
101	**TLB 600**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
102	**TLB 601**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
103	**TLB 602**	**+**	**+**	**+**	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**
Total	**(%)**	**90**	**100**	**98**	**78**	**100**	**99**	**96**	**91**	**98**	**88**	**83**	**96**	**21**	**64**	**28**	**98**	**51**

ARB, Arabinose; CLB, Cellobiose; FRU, Fructose; GAL, Galactose; GLU, Glucose; LAC, Lactose; MAL, Maltose; MAN, Mannose; MNT, Mannitol, MLZ, Melizitose; MLB, Melibiose; RAF, Raffinose; RHM, Rahminose; SAL, Salicine; SRB, Sorbitol; SUC, Sucrose; XYL, xylose; +, positive; -, negative; wp, weak positive.

6.3. Assessment of Probiotic Properties of Lactobacilli

6.3.1. Biochemical Tests

For the primary identification of selected 14 *Lactobacillus* isolates, biochemical tests listed in Bergey's manual (97) as reference for the classification of *Lactobacillus* were followed. The result of biochemical tests indicated that all isolates were immobile, negative for IMVIC tests which were used to identify and classify the pathogenic strain of *Enterobacteriacea*. All *Lactobacillus* isolates strains were heterofermentative as indicated by the absence of gas production from glucose and negative for gelatinase production

6.3.2. Growth under Different NaCl Concentrations

All 14 isolates were able to grow in presence of 1 to 7 % NaCl, but not above 8% (Table 4).

6.4. Resistance to Acidity

It was found that 29 out of 103 *Lactobacillus* were able to grow under different pH values starting from 6.2, 5.0, 4.0, 3.0, and 2.0. The results indicated that isolates TLB 519, TLB 520, TLB 582, and TLB 590 were the most resistance strains which able to grow under pH 2 and 3. Commercial yogurt isolates observed better acidity resistance values in comparison to other dairy isolates (Table 5).

Table 4. Growth of *Lactobacillus* strains in the presence of different NaCl concentrations.

Strains	1%	2 %	3 %	4 %	5 %	6 %	7 %	8 %	9 %	10 %
TLB 504	+	+	+	+	+	+	+	-	-	-
TLB 506	+	+	+	+	+	+	+	-	-	-
TLB 509	+	+	+	+	+	+	+	-	-	-
TLB 513	+	+	+	+	+	+	+	-	-	-
TLB 514	+	+	+	+	+	+	+	-	-	-
TLB 519	+	+	+	+	+	+	+	-	-	-
TLB 520	+	+	+	+	+	+	+	-	-	-
TLB 521	+	+	+	+	+	+	+	-	-	-
TLB 543	+	+	+	+	+	+	+	-	-	-
TLB 544	+	+	+	+	+	+	+	-	-	-
TLB 554	+	+	+	+	+	+	+	-	-	-
TLB 580	+	+	+	+	+	+	+	-	-	-
TLB 582	+	+	+	+	+	+	+	-	-	-
TLB 590	+	+	+	+	+	+	+	-	-	-

+ , growth present; - , no growth

Table 5. Acidity tolerance of isolated *Lactobacillus* strains.

No	Strain	pH 6.5	pH 5.0	pH 4.0	pH 3.0	pH 2.0
1	TLB 502	2.67±0.06	2.00±0.13	0.6±0.04	0.334±0.06	0.202±0.03
2	TLB 503	2.56±0.04	2.10±0.09	0.48±0.15	0.333±0.03	0.207±0.02
3	TLB 504	2.75±0.13	2.38±0.20	0.549±0.09	0.334±0.08	0.21±0.03
4	TLB 506	2.75±0.04	2.43±0.03	0.562±0.09	0.321±0.03	0.185±0.06
5	TLB 509	2.32±0.07	1.77±0.17	0.619±0.10	0.351±0.05	0.196±0.04
6	TLB 513	2.54±0.07	2.41±0.02	0.617±0.07	0.376±0.01	0.225±0.01
7	TLB 514	2.57±0.02	2.17±0.16	0.607±0.09	0.360±0.06	0.231±0.01
8	TLB 515	2.84±0.10	2.38±0.09	0.58±0.06	0.310±0.05	0.231±0.01
9	TLB 516	2.65±0.03	2.43±0.14	0.557±0.08	0.288±0.07	0.305±0.05
10	TLB 517	2.3±0.24	2.21±0.10	0.649±0.07	0.483±0.06	0.211±0.02
11	TLB 519	2.47±0.03	2.22±0.03	0.642±0.07	0.581±0.02	0.485±0.03
12	TLB 520	2.71±0.14	1.96±0.05	0.638±0.08	0.517±0.02	0.391±0.05
13	TLB 521	2.36±0.02	1.79±0.11	0.602±0.08	0.471±0.02	0.221±0.02
14	TLB 541	2.13±0.01	1.72±0.03	0.571±0.07	0.297±0.04	0.209±0.03
15	TLB 543	2.23±0.06	1.84±0.08	0.626±0.08	0.333±0.02	0.196±0.04
16	TLB 544	2.16±0.05	1.70±0.16	0.541±0.10	0.359±0.02	0.201±0.03
17	TLB 554	2.16±0.02	1.78±0.13	0.627±0.11	0.386±0.02	0.303±0.07

Tablo 5. (Continued)

No	Strain	pH 6.5	pH 5.0	pH 4.0	pH 3.0	pH 2.0
18	TLB 566	2.31±0.05	2.11±0.04	0.666±0.06	0.332±0.02	0.207±0.02
19	TLB 580	2.17±0.05	1.81±0.12	0.668±0.04	0.456±0.04	0.230±0.02
20	TLB 581	2.17±0.06	1.84±0.05	0.641±0.04	0.332±0.03	0.215±0.01
21	TLB 582	2.18±0.05	2.12±0.18	0.577±0.06	0.326±0.02	0.415±0.33
22	TLB 583	2.12±0.02	1.8±0.10	0.604±0.04	0.337±0.02	0.209±0.03
23	TLB 584	2.13±0.02	1.77±0.14	0.629±0.03	0.335±0.02	0.215±0.02
24	TLB 586	2.11±0.02	1.59±0.13	0.638±0.04	0.328±0.03	0.214±0.02
25	TLB 588	2.21±0.09	1.98±0.18	0.564±0.03	0.320±0.02	0.204±0.02
26	TLB 589	2.11±0.07	1.68±0.09	0.555±0.02	0.329±0.03	0.199±0.03
27	TLB 590	2.55±0.06	1.9±0.09	0.612±0.02	0.441±0.06	0.332±0.01
28	TLB 591	2.11±0.12	1.92±0.06	0.579±0.07	0.388±0.02	0.193±0.01
29	TLB 600	2.22±0.09	1.79±0.10	0.565±0.07	0.365±0.02	0.209±0.02

Results are expressed as mean ± SD with three separate experiments.

6.5. Resistance to Bile Salt

Only the strains that proved resistance for different acidity concentration was selected for bile salt resistance determination. Fourteen out of 29 isolates which observed resistant to bile salt concentration were selected, and strain TLB 513 was the most resistant one which was able to tolerate 0.3% bile salt for 6 h. The result also indicated that the resistance levels decreased as bile salt concentration increased. Commercial yogurt isolates observed better bile salts resistance value in comparison to other dairy isolates (Table 6, Figures 6, 7, and 8).

Table 6. Resistance of *Lactobacillus* strains to different bile salt concentrations

Strain	0.3 % Bile			0.15 % Bile			0.06 % Bile		
	0 h	3 h	6 h	0 h	3 h	6 h	0 h	3 h	6 h
TLB 502	0.55±0.01	0.90±0.13	0.96±0.04	0.54±0.04	0.96±0.03	1.22±0.07	0.5±0.15	1.51±0.10	1.26±0.17
TLB 503	0.29±0.13	0.44±0.08	0.53±0.09	0.28±0.12	1.04±0.18	1.36±0.49	0.27±0.11	1.54±0.78	2.29±1.09
TLB 504	0.52±0.01	0.81±0.24	1.06±0.31	0.511±0.011	0.93±0.06	1.20±0.17	0.53±0.06	1.51±0.04	2.55±0.30
TLB 506	0.56±0.01	0.92±0.14	1.03±0.14	0.536±0.01	1.04±0.10	1.20±0.25	0.52±0.02	1.48±0.10	2.23±0.23
TLB 509	0.50±0.03	0.82±0.03	0.86±0.09	0.5±0.026	0.79±0.17	1.06±0.14	0.48±0.014	1.09±0.23	1.97±0.23
TLB 513	0.46±0.06	1.09±0.17	1.22±0.20	0.44±0.061	1.31±0.10	1.53±0.07	0.44±0.063	1.64±0.07	2.48±0.19
TLB 514	0.58±0.06	0.88±0.10	1.06±0.11	0.58±0.036	0.98±0.13	1.27±0.19	0.55±0.045	1.49±0.07	2.51±0.26
TLB 515	0.49±0.06	0.54±0.08	0.79±0.15	0.49±0.05	0.74±0.22	1.03±0.16	0.49±0.05	1.03±0.20	2.09±0.42
TLB 516	0.52±0.04	0.64±0.14	0.71±0.14	0.51±0.049	0.73±0.12	0.99±0.06	0.51±0.06	1.03±0.19	2.15±0.50
TLB 517	0.52±0.05	0.80±0.17	0.99±0.06	0.5±0.072	0.91±0.39	1.13±0.29	0.5±0.07	1.30±0.14	2.44±0.40
TLB 519	0.58±0.07	0.81±0.06	0.89±0.01	0.51±0.04	0.96±0.08	1.16±0.20	0.57±0.04	1.43±0.18	2.18±0.05
TLB 520	0.55±0.02	0.87±0.03	1.21±0.34	0.52±0.026	1.07±0.20	1.26±0.32	0.53±0.02	1.43±0.08	2.38±0.32

Tablo 6. (Continued)

Strain	0.3 % Bile			0.15 % Bile			0.06 % Bile		
	0 h	3 h	6 h	0 h	3 h	6 h	0 h	3 h	6 h
TLB 521	0.48±0.06	1.10±0.14	1.18±0.06	0.46±0.044	1.41±0.07	1.71±0.12	0.45±0.05	1.62±0.08	2.55±0.22
TLB 541	0.56±0.04	0.82±0.09	0.93±0.10	0.55±0.040	1.00±0.12	1.22±0.24	0.58±0.10	1.44±0.04	2.51±0.27
TLB 543	0.46±0.00	1.02±0.14	1.09±0.14	0.44±0.005	0.64±0.52	0.79±0.59	0.40±0.011	0.83±0.72	1.41±1.16
TLB 544	0.45±0.06	1.02±0.05	1.11±0.03	0.43±0.05	1.15±0.21	1.27±0.30	0.47±0.046	1.35±0.27	2.05±0.49
TLB 554	0.35±0.10	1.03±0.24	1.16±0.32	0.34±0.1	1.01±0.14	1.02±0.11	0.33±0.11	1.02±0.17	1.35±0.21
TLB 566	0.46±0.07	0.90±0.08	1.04±0.09	0.44±0.07	1.23±0.04	1.54±0.08	0.42±0.066	1.52±0.08	2.61±0.11
TLB 580	0.47±0.05	0.91±0.13	1.00±0.15	0.45±0.04	1.23±0.03	1.45±0.08	0.44±0.03	1.46±0.08	2.55±0.09
TLB 581	0.47±0.03	0.91±0.03	1.01±0.08	0.44±0.03	1.24±0.02	1.46±0.09	0.41±0.011	1.45±0.14	2.57±0.13
TLB 582	0.31±0.13	0.71±0.07	0.82±0.07	0.29±0.11	1.06±0.14	1.22±0.14	0.28±0.10	0.98±0.24	1.81±0.80
TLB 583	0.35±0.07	0.53±0.06	0.77±0.09	0.25±0.09	0.92±0.06	1.01±0.10	0.24±0.09	0.69±0.02	0.91±0.09
TLB 584	0.28±0.06	0.59±0.08	0.66±0.03	0.25±0.04	0.94±0.08	1.01±0.05	0.24±0.05	0.82±0.05	1.68±0.86
TLB 586	0.43±0.07	0.73±0.03	0.78±0.07	0.40±0.05	1.38±0.05	1.57±0.11	0.39±0.05	1.75±0.25	2.50±0.26
TLB 588	0.31±0.09	0.72±0.26	0.92±0.36	0.30±0.076	1.27±0.23	1.54±0.45	0.29±0.07	1.67±0.23	2.58±0.26
TLB 589	0.16±0.01	0.38±0.13	0.55±0.30	0.15±0.01	0.88±0.06	0.77±0.05	0.14±0.008	0.69±0.08	1.00±0.24
TLB 590	0.27±0.15	0.70±0.23	0.83±0.36	0.26±0.13	1.16±0.26	1.52±0.24	0.25±0.13	1.52±0.14	2.46±0.41
TLB 591	0.21±0.10	0.52±0.04	0.65±0.15	0.20±0.09	0.92±0.22	1.14±0.30	0.20±0.009	0.94±0.25	1.51±0.86
TLB 600	0.22±0.15	0.40±0.25	0.44±0.21	0.20±0.14	0.99±0.28	1.13±0.49	0.20±0.133	1.06±0.46	1.84±0.96

Result are expressed as mean ± SD with three separate experiments.

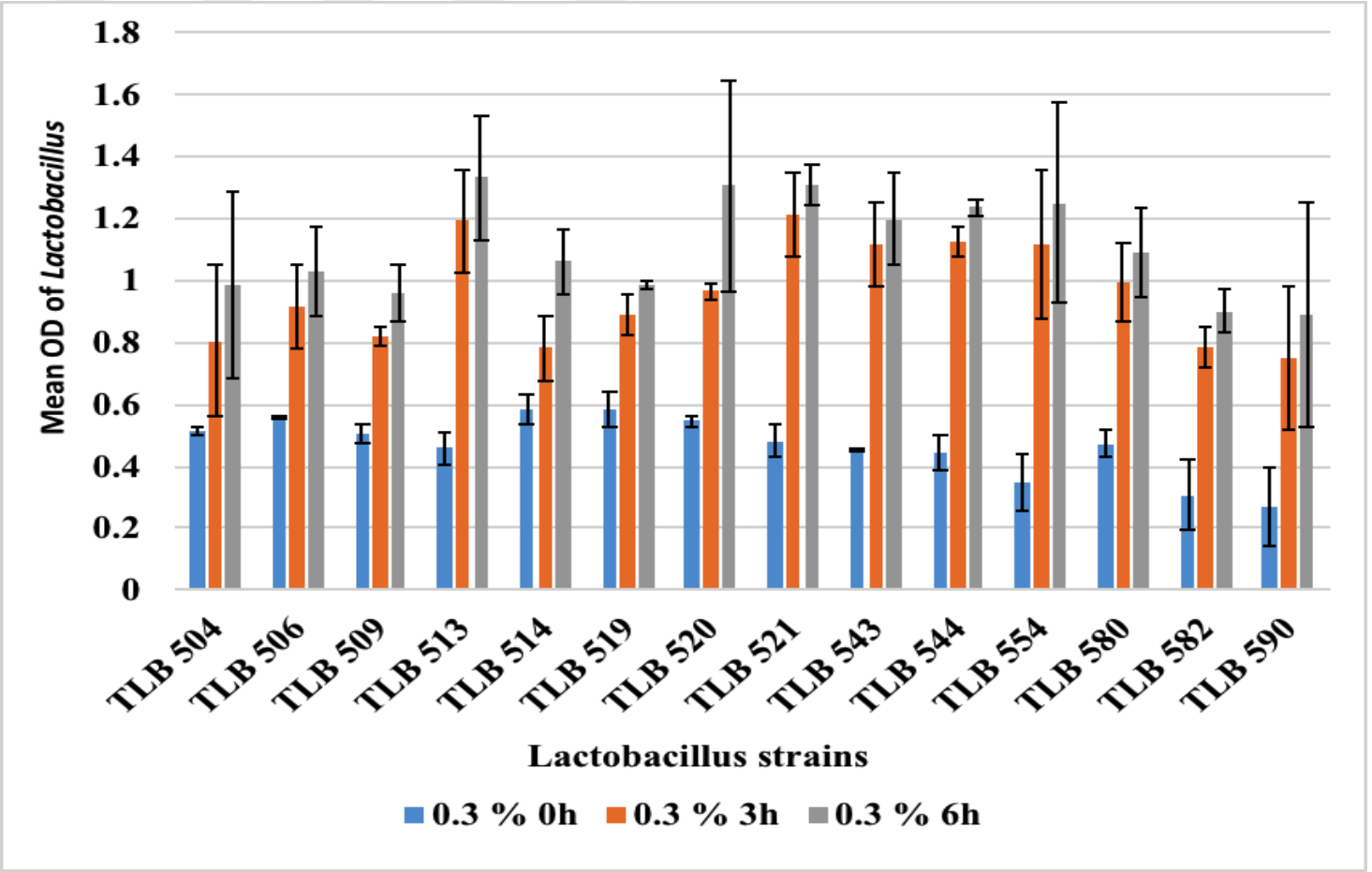

Figure 6. Resistance of *Lactobacillus* to 0.3% bile salt for different time periods.

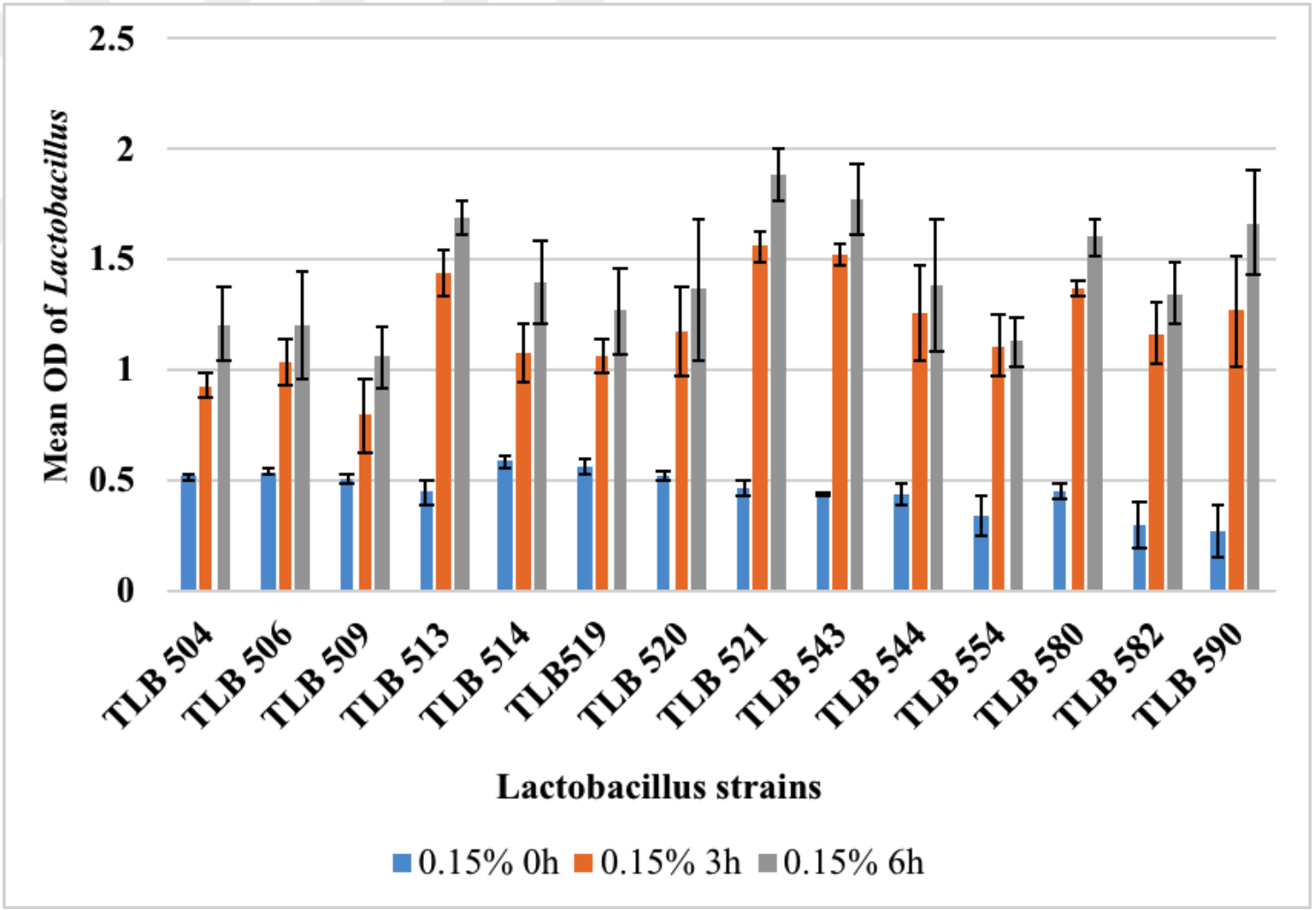

Figure 7. Resistance of *Lactobacillus* to 0.15% bile salt for different time periods.

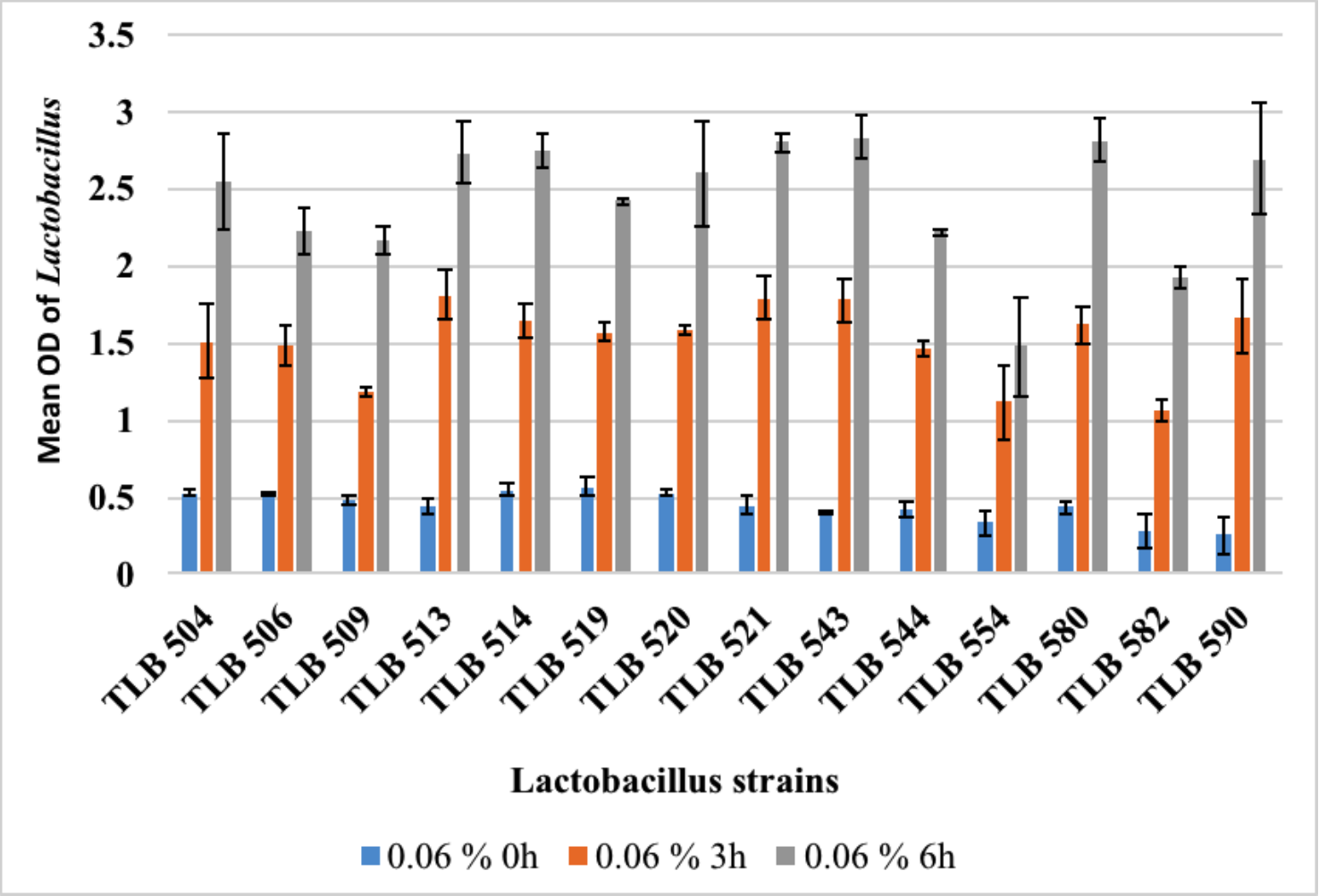

Figure 8. Resistance of *Lactobacillus* to 0.06% bile salt for different time periods.

6.6. Pepsin and Pancreatin Resistance

Selected 14 *Lactobacillus* strains showed different resistance rates to the treatmentment with 0.3% pepsin and 0.1% pancreatin for various time periods. The strain TLB 580 was the most resistance strain which showed 86% and 97.7 % growth rates, respectively, in the presence of these two enzymes. The tests were performed in triplicate by measuring the viable cell count in terms of CFU mL^{-1} (Table 7 and 8).

Table 7. Survival of *Lactobacillus* strain in the presence of 3% pepsin

Strains	**CFU/mL (t_0 h)**	**CFU/mL (t_3 h)**	**Survival rate (%)**
TLB 504	3.88×10^{10}	4.07×10^{10}	62.4
TLB 506	1.56×10^{10}	4.45×10^{7}	74.9
TLB 509	2.19×10^{10}	1.0×10^{6}	60.0
TLB 513	1.98×10^{10}	1.0×10^{6}	58.6
TLB 514	2.6×10^{9}	4.0×10^{6}	70.0
TLB 519	6.4×10^{10}	1.96×10^{6}	58.2
TLB 520	1.5×10^{10}	2.3×10^{5}	52.0
TLB 521	2.58×10^{10}	3.23×10^{6}	62.0
TLB 543	6.9×10^{10}	3.15×10^{6}	69.0
TLB 544	3.79×10^{10}	4.62×10^{6}	63.0
TLB 554	1.95×10^{10}	3.19×10^{6}	63.0
TLB 580	6.46×10^{10}	2.12×10^{9}	86.0
TLB 582	3.23×10^{10}	2.01×10^{5}	50.0
TLB 590	5.47×10^{10}	2.65×10^{6}	56.0

Results are expressed as mean ± SD with three separate experiments.

Table 8. Survival rates *of Lactobacillus* strains in the presence of 1% pancreatin

Strain	CFU/mL (t_0 h)	CFU/mL (t_4 h)	Survival rate (%)
TLB 504	3.8×10^{10}	1.88×10^{10}	97.0
TLB 506	3.71×10^{10}	1.9×10^{10}	96.0
TLB 509	5.23×10^{10}	1.49×10^{10}	94.0
TLB 513	5.13×10^{10}	1.46×10^{10}	94.0
TLB 514	5.23×10^{10}	4.9×10^{10}	90.0
TLB 519	4.14×10^{10}	4.0×10^{9}	90.0
TLB 520	2.88×10^{10}	3.95×10^{9}	91.0
TLB 521	2.7×10^{10}	1.82×10^{10}	98.0
TLB 543	9.7×10^{10}	1.23×10^{10}	91.0
TLB 544	5.0×10^{10}	6.9×10^{9}	91.0
TLB 554	2.8×10^{10}	9.6×10^{9}	95.5
TLB 580	4.7×10^{10}	2.74×10^{10}	97.7
TLB 582	3.7×10^{10}	6.3×10^{9}	92.6
TLB 590	4.6×10^{10}	8.36×10^{9}	92.9

Results are expressed as mean ± SD with three separate experiments.

6.7. Hemolytic Activity

Five out of 14 *Lactobacillus* strains, TLB 519, TLB 520, TLB 521, TLB 543, TLB 544 showed alpha hemolysis and remaining nine strains had gamma (no) hemolysis (Table 9 and Figure 9).

Table 9. Haemolytic activities of the *Lactobacillus* strains

***Lactobacillus* strains**	**Hemolysis**
TLB 504, TLB 506, TLB 509, TLB 513, TLB 514, TLB 554, TLB 580, TLB 582, TLB 590	Gamma (γ)
TLB 519, TLB 520, TLB 521, TLB 543, TLB 544	Alpha (α)

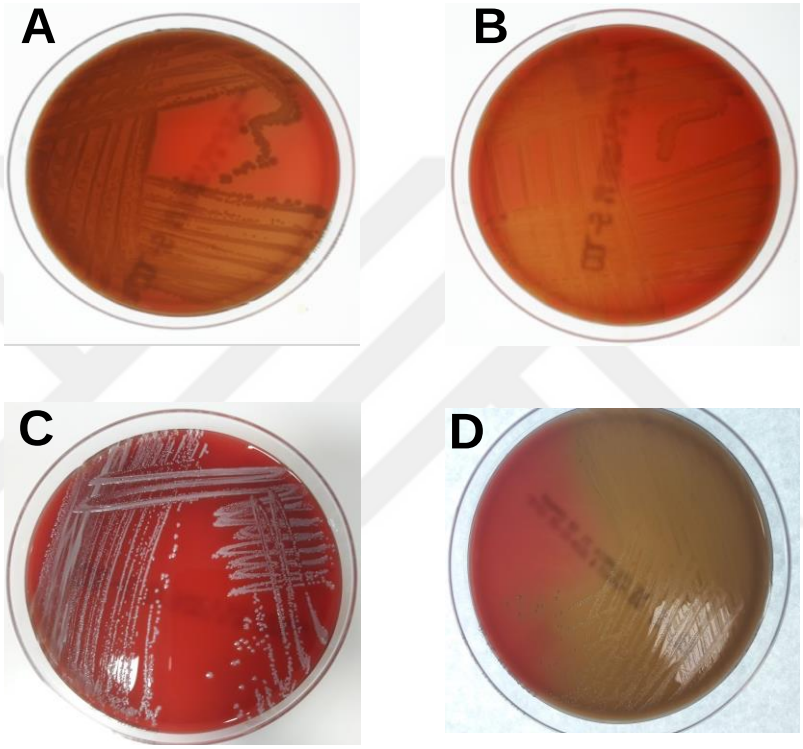

Figure 9. Haemolytic activity of selected bacterial species. A, S. *aureus* ATCC 25923 (beta haemolysis); B, *E. coli* ATCC 25292 (alpha haemolysis); C, *E. faecium* ATCC 6057 (gamma haemolysis); D, *Lactobacillus* strain TLB 519 (alpha haemolysis).

6.8. Antibiotic Susceptibility of Selected *Lactobacillus* Strains

All 14 strains were found to be resistant to three antibiotics including ciprofloxacin, teicoplanin and vancomycin, and susceptible to amoxicillin, azithromycin, chloramphenicol, erythromycin, penicillin, and streptomycin (Table 10 and Figure 10).

Table 10. Results of antibiotic susceptibility tests

Strain	AX 30 µg	AZM 15 µg	C 30 µg	E 15 µg	CN 10 µg	CIP 5 µg	P 10 unit	ST 10 µg	TEC 30 µg	VA 30 µg
TLB 504	S	S	S	S	S	R	S	S	R	R
TLB 506	S	S	S	S	S	R	S	S	R	R
TLB 509	S	S	S	S	S	R	S	S	R	R
TLB 513	S	S	S	S	S	R	S	S	R	R
TLB 514	S	S	S	S	S	R	S	S	R	R
TLB 519	S	S	S	S	S	R	S	S	R	R
TLB 520	S	S	S	S	S	R	S	S	R	R
TLB 521	S	S	S	S	S	R	S	S	R	R
TLB 543	S	S	S	S	S	R	S	S	R	R
TLB 544	S	S	S	S	S	R	S	S	R	R
TLB 554	S	S	S	S	S	R	S	S	R	R
TLB 580	S	S	S	S	S	R	S	S	R	R
TLB 582	S	S	S	S	S	R	S	S	R	R
TLB 590	S	S	S	S	S	R	S	S	R	R

R, Resistant; S, Sensitive; AX, Amoxicillin; AZM, Azithromycin; C, Chloramphenicol; E, Erythromycin; CN, Gentamycine; CIP, Ciprofloxacin; P, Penicillin; ST, Streptomycin; TEC, Teicoplanin; VA, Vancomycin.

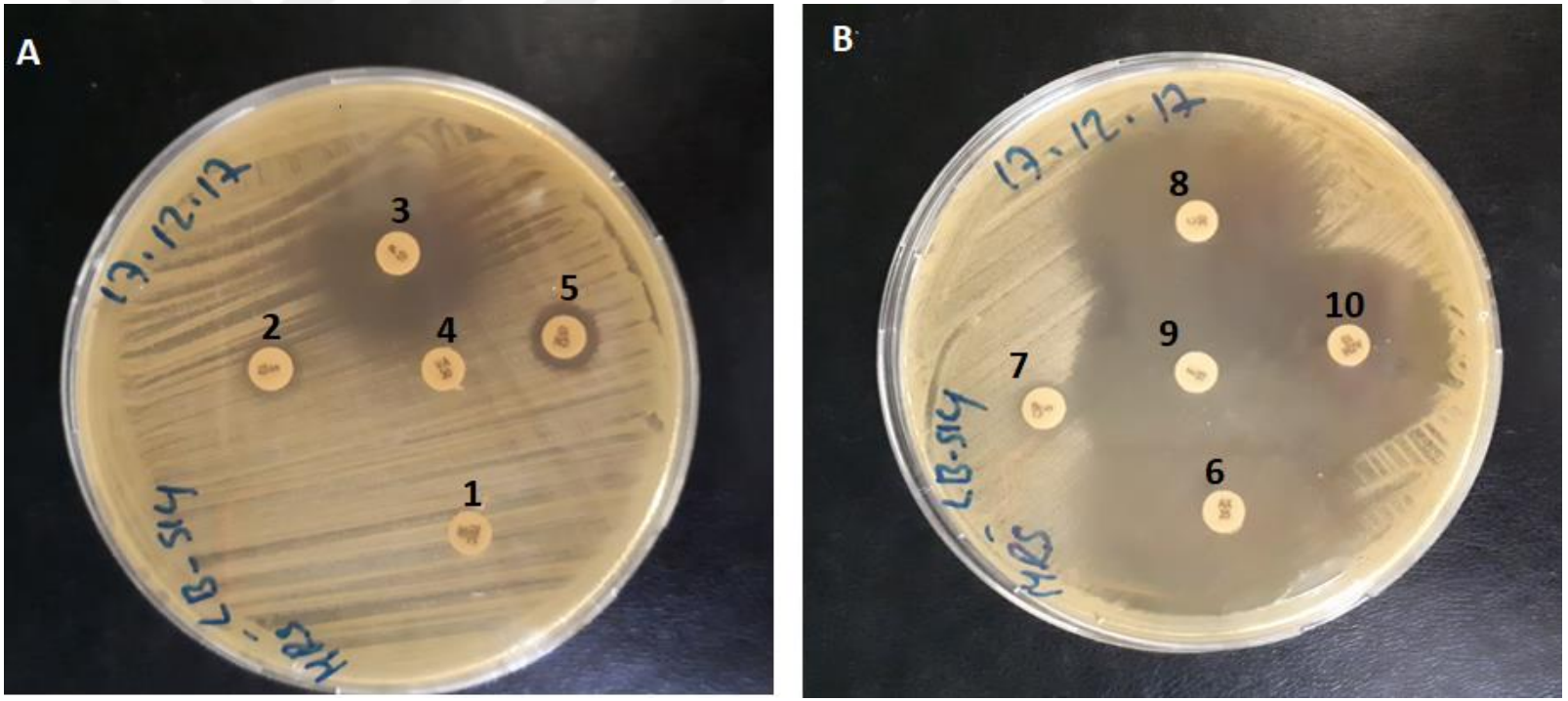

Figure 10. Antibiotic sensitivity test results of *Lactobacillus* strain TLB 514. 1, Teicoplanin; 2, Streptomycin; 3, Penicillin;, Vancomycine; 5, Gentamycine; 6, Amoxicillin; 7, Ciprofloxacine; 8, Chloramphenicol; 9, Erythromycine; 10, Azithromycin.

6.9. Adhesion Index and Adhesion Score Determination

The results of the adhesion index (AI) test indicated that six of 14 of *Lactobacillus* strains, TLB 509, TLB 506, TLB 504, TLB 513, TLB 520, TLB 521, showed high adhesion indexes with 79.23%, 78.7%, 78.3%, 74.1%, 73.18%, 73.01%, respectively. These six strains were subjected to further adhesion test to determine their adhesion ability of non-adhesive, adhesive and strongly adhesive. Based on the Gram staining, TLB 590 was the most strongly adhesive strains (102±1.4) while the other 5 strains scored as adhesive strains (Table 11 and Figure 11).

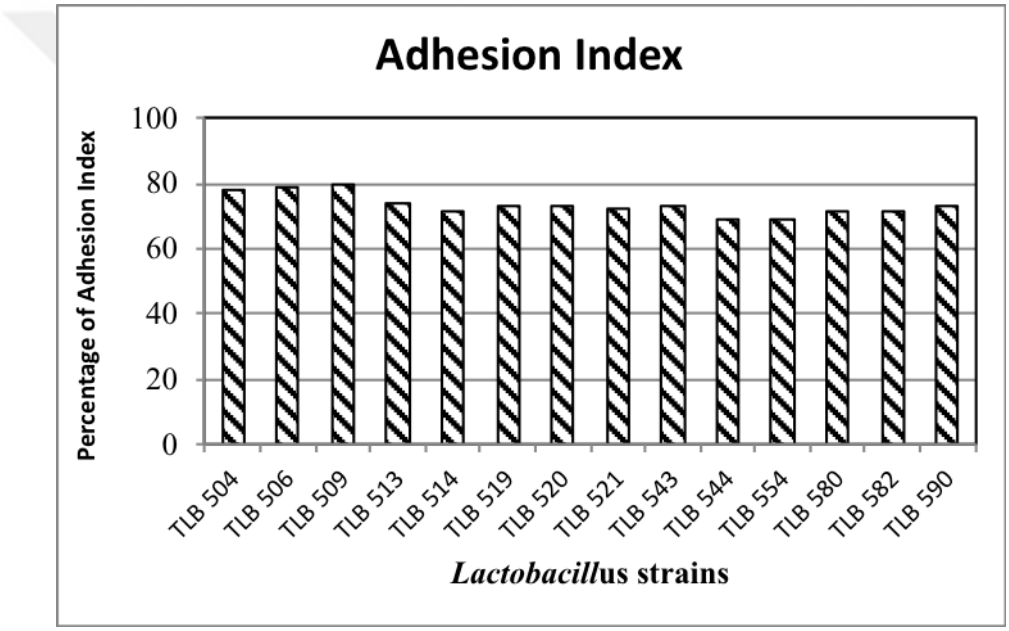

Figure 11. In vitro adhesion index of selected *Lactobacillus* strains

Table 11. Adhesion index of six *Lactobacillus* Strains

Strains	Total adherent bacteria	Interpretation
TLB 504	88±1.256	Adhesive strain
TLB 506	98±1.5	Adhesive strain
TLB 509	93±1.35	Adhesive strain
TLB 513	96 ±1.84	Adhesive strain
TLB 519	93 ±1.15	Adhesive strain
TLB 590	102±1.4	Strongly adhesive strain

Results are expressed as mean±SD with three separate experiments.

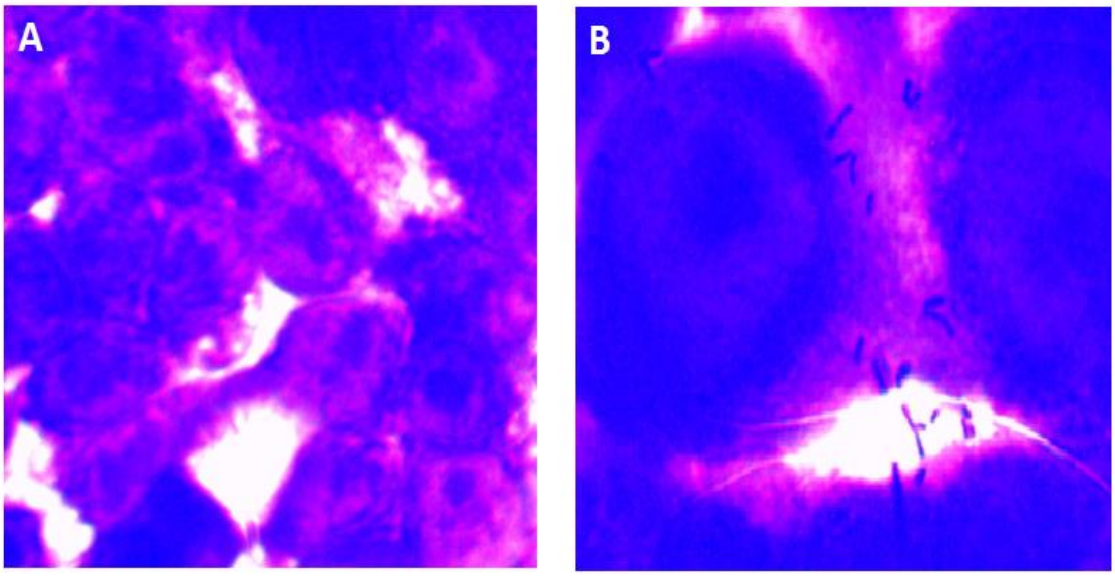

Figure 12. Adhesion score Index of six selected *Lactobacillus* evaluated by Gram staining method. A, HT-29 cell without staining; B, HT-29 cell stained with (0.5 %) crystal violet showing adhered bacteria.

6.10. Characterization of Antimicrobial Compounds Produced by *Lactobacillus* Strains

Supernatants of 14 *Lactobacillus* strains were evaluated for the presence of antimicrobial compounds against a number of of pathogens including *E. coli* ATCC 29212, *E. faecalis* ATCC 29212, *S. pyogen* ATCC 12344, *K. pneumonia* ATCC 13883, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *S. typhi* ATCC 14028, *P.*

mirabilis ATCC 13315, and *C. albicans* ATCC 10231 by agar well diffusion method. Interpretations of the results are shown in Table l2. Antimicrobial activity against *E. coli* ATCC 29212 was due to production of organic acid while antimicrobial activity against *E. faecalis* ATCC 29212 and *S. pyogen* ATCC 12344 were due to production of both H_2O_2 and organic acids (Table 13, 14, 15 and Figure 13). Except these three pathogens, other tested pathogens were not inhibited by the byproducts of *Lactobacillus* strains.

Table 12. Antimicrobial activity results of *Lactobacillus* strains

Supernatant treatment	**Inhibition zone Presence or absence**	**Interpretation of antagonistic substance**
Non-treated	Present	Organic acids H_2O_2 or bacteriocins
	Absent	Bacteriocins
Trypsin treated	Present	Organic acids H_2O_2
Catalase treated	Absent	H_2O_2
Neutrilized with NaOH	Present	Bacteriocin or H_2O_2
	Absent	Organic acids

Table 13. Inhibitory activities of *Lactobacillus* strains against *E. coli* ATCC 25292

Strains	Untreated supernatant	Trypsin treated Supernatant	Catalase treated Supernatant	Neutralized supernatant
TLB 504	14.3 ±0.57	14.0±0	13.66±1.15	-
TLB 506	14.6±0.58	15.33±0.58	14.0±1.0	-
TLB 509	14.6±0.57	15.66±0.58	13.33±1.5	-
TLB 513	13.6±0.58	13.33±0.58	12.66±0.58	-
TLB 514	14.3±1.15	15.33±0.58	13.66±0.58	-
TLB 519	15.3±1.15	14.66±0.58	14.0±2.0	-
TLB 520	15.66±1.52	15.33±0.58	13.33±1.5	-
TLB 521	14.33±1.15	14.0±1.0	12.66±0.58	-
TLB 543	14.0±1.0	13.66±0.58	14.0±1.0	-
TLB 544	14.0±1.0	14.0±1.0	14.66±1.52	-
TLB 554	16.0±0.58	14.33±1.15	15.33±0.58	-
TLB 580	14.0±0.7	14.0±1.0	15.0±1.0	-
TLB 582	12.6±0.58	13.66±1.53	14.66±1.52	-
TLB 590	14.6±0.58	13.66±0.58	13.66±1.15	-

-, no- inhibition

Table 14. Inhibitory activity of *Lactobacillus* isolates against *E. faecalis* ATCC 29212

Strains	Untreated supernatant	Trypsin treated Supernatant	Catalase treated Supernatant	Neutralized supernatant
TLB 504	16.0±1.0	11.66±0.57	-	-
TLB 506	16.33±1.154	12.66±0.57	-	-
TLB 509	17.0±1.0	12.33±0.57	-	-
TLB 513	16.33±0.58	10.0±0.0	-	-
TLB 514	16.66±1.52	12.0±1.73	-	-
TLB 519	17.0±1.0	11.33±1.154	-	-
TLB 520	17.66±0.58	11.0±1.0	-	-
TLB 521	16.33±0.58	10.0±0.0	-	-
TLB 543	15.66±0.58	9.66±0.58	-	-
TLB 544	15.33±1.15	10.33±1.54	-	-
TLB 554	14.66±1.15	11.33±1.154	-	-
TLB 580	14.0±0	11.66±0.577	-	-
TLB 582	14.33±0.57	9.66±0.577	-	-
TLB 590	15.33±0.577	10.33±1.54	-	-

-, no- inhibition

Table 15. Inhibitory activity of *Lactobacillus* strains against *S. pyogen* ATCC 29212

Strains	Untreated supernatant	Trypsin treated Supernatant	Catalase treated Supernatant	Neutralized supernatant
TLB 504	15.0±1.0	12.66±0.57	-	-
TLB 506	15.33±1.154	11.66±0.57	-	-
TLB 509	16.0±1.0	13.33±0.57	-	-
TLB 513	16.33±0.57	11.0±1.0	-	-
TLB 514	15.66±1.52	12.0±1.73	-	-
TLB 519	15.0±1.0	11.33±1.154	-	-
TLB 520	16.66±0.58	11.0±1.0	-	-
TLB 521	15.33±0.58	10.0±1.0	-	-
TLB 543	16.66±0.58	9.66±0.58	-	-
TLB 544	14.33±1.15	10.33±1.54	-	-
TLB 554	15.66±1.15	11.33±1.154	-	-
TLB 580	15.0±0.0	11.66±0.577	-	-
TLB 582	14.33±0.57	9.66±0.577	-	-
TLB 590	16.33±0.577	10.33±1.54	-	-

-, no- inhibition

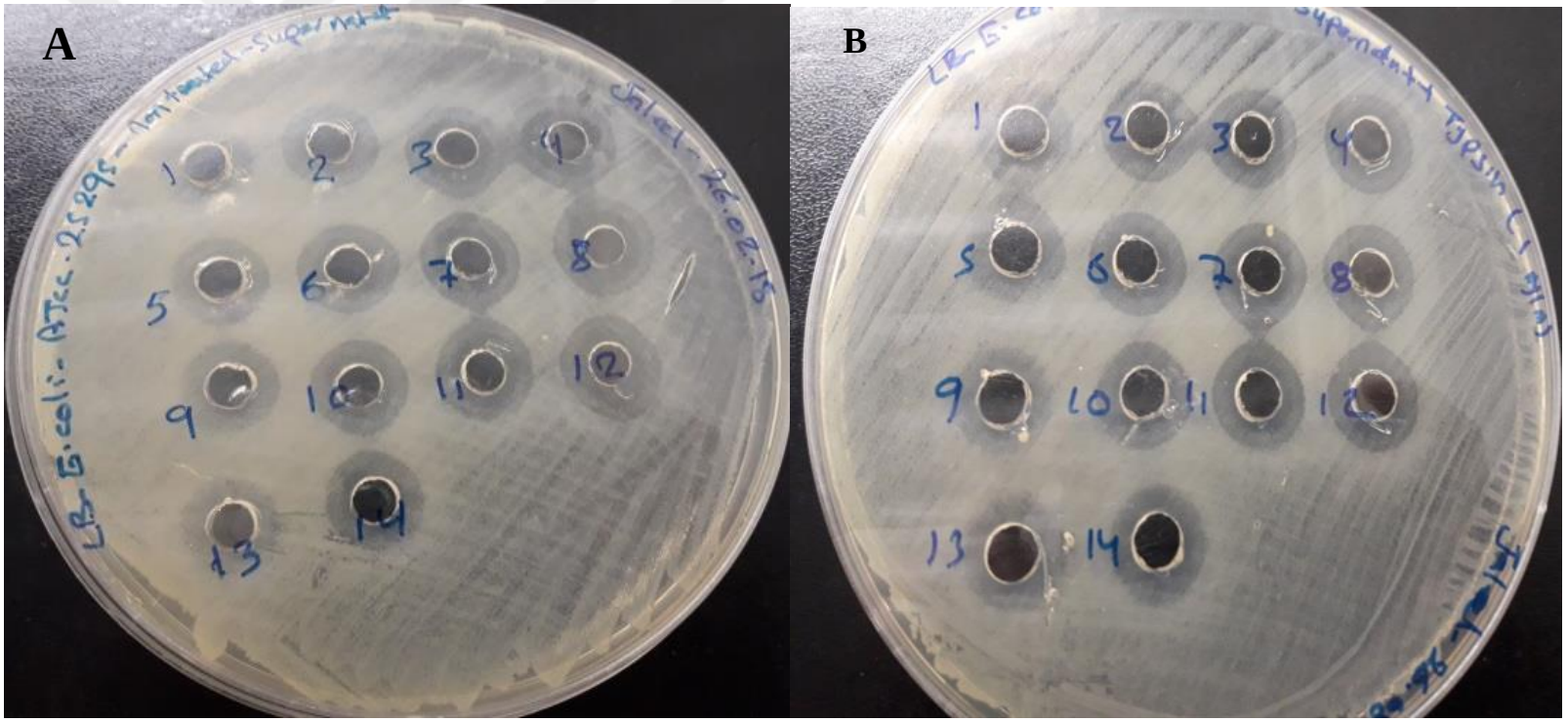

Figure 13. Inhibitory Activity of selected *Lactobacillus* isolates against *E. coli* ATCC 25292 A, non-treated supernatant; B, zone inhibition result of supernatant treated with catalase (0.5 mg/mL). 1, TLB 504; 2, TLB 506; 3, TLB 509; 4, TLB 513;5, TLB 514; 6, TLB 519; 7, TLB 520; 8, TLB 521; 9, TLB 543; 10, TLB 544; 11, TLB 554; 12, TLB 580; 13, TLB 582; 14, TLB 590.

6.11. Characterization of *Lactobacillus* for Presence of Bcteriophage and Bacteriocin Production

Cell lysetes of each *Lactobacillus* strain produced by mitomycin C induction were filtered and their pH were adjusted to 5.5 to 6.5. Each lysate was dropped on MRS soft agar seeded with all 14 *Lactobacillus* strains. None of the strains produced phage plaques or lysis zone with the tested indicator strains.

6.12. Screening for Biosurfactant Production

6.12.1. Oil Spreading Test

Three types of oil were used as substrates for detection of cell-bound biosurfactants, and distilled water as negative control. All 14 strain were able to produce biosurfactants which were indicated by high score area of spreading when mixed with three different types of oils such as kerosene, olive oil, and sun flower oil. All the strains showed high score of spreading when treated with kerosene oil, especially TLB 544, which scored a diameter of 35.33±0.58 (Table 16).

6.12.2. Drop Collapse Test

The ability of *Lactobacillus* to produce cell-bound biosurfactants was identified by plate-well method. Results indicated that the strains TLB 504, TLB 506, TLB 509, TLB 513, TLB 544, and TLB 590 were the only stains produced cell-bound biosurfactants (Table17 and Figure 14).

6.12.3. Emulsification Index (EI)

The ability of *Lactobacillus* strains to produce cell-bound biosurfactant was also conducted by observing their ability to emulsify two immiscible fluids, water and three different types of oils as substrates. All 14 strains showed high (EI) under sunflower oil, while mostly observed less (EI) under kerosene treatment (Table 18 and Figure 15). The results of these three tests led to the selection of six strains, TLB 504, TLB 506, TLB 509, TLB 513, TLB 544, and TLB 590 as biosurfactant producers. These six strains were further tested for their antimicrobial activities against different pathogenic bacteria.

Table16. Biosurfactant Production of Lactobacillus strains by Oil Spreading Test

Strain	Diameter of oil spreading (mm)		
	Olive Oil	**Sun Flower Oil**	**Kerosene Oil**
TLB504	7.67±0.58	11.33±0.58	28.67±0.58
TLB 506	7.00±1.73	9.67±0.58	32.33±2.31
TLB 509	6.67±1.15	9.00±1.00	31.67±2.89
TLB 513	6.67±1.53	9.67±0.58	29.33±5.51
TLB 514	6.33±1.15	9.67±0.58	34.67±4.73
TLB 519	6.33±2.31	10.33±0.58	33.33±7.64
TLB 520	4.67±0.58	7.67±0.58	34.67±0.58
TLB 521	4.67±0.58	7.67±0.58	30.67±3.79
TLB 543	4.33±0.58	10.67±0.58	25.00±1.73
TLB 544	6.33±1.15	7.67±1.53	35.33±0.58
TLB 554	7.33±0.58	4.67±0.58	27.67±1.15
TLB 580	6.67±0.58	7.33±1.15	29.67±4.62
TLB 582	6.00±1.00	9.33±1.53	34.67±0.58
TLB 590	7.67±1.53	11.33±1.15	34.67±0.58

Values are expressed as mean ± SD with three separate experiments.

Table 17. Drop collapse determination test

Strains	Results
TLB 504	+
TLB 506	+
TLB 509	+
TLB 513	+
TLB 514	-
TLB 519	-
TLB 520	-
TLB 521	-
TLB 543	-
TLB 544	+
TLB 554	-
TLB 580	-
TLB 582	-
TLB 590	+*

-, no drop collapse activity; +*, drop collapse observed within 1 min; +, drop collapse observed after 3 min of biosurfactant addition.

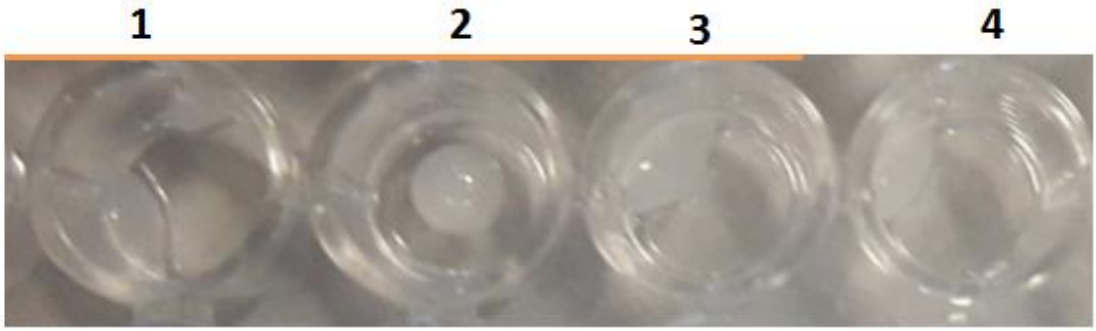

Figure 14. Drop collapse determination test 1, positive control result Triton X-100 (0.1 %); 2, negative control results deionized water; 3, result of TLB 504 strain; 4, result of TLB 506.

Table 18. Emulsification index (EI) of *Lactobacillus* strains

	E24 (I %) value		
Strains	**Kerosene oil**	**Olive Oil**	**Sunflower oil**
TLB 504	3.2/0.5 (15.62)	3.1/0.6 (16.1)	3.3/0.7 (21.21)
TLB 506	3.5/0.6 (17.14)	3.4/0.7 (20.4)	3.4/0.8 (23.5)
TLB 509	3.3/0.5 (15.15)	3.4/0.4 (17.64)	3.2/0.7 (21.87)
TLB 513	3.5/0.5 (15.15)	3.4/0.8 (23.5)	3.2/0.8 (25)
TLB 514	3.5/0.4 (11.42)	3.3/0.5 (15.15)	3.2/0.6 (18.75)
TLB 519	3.2/0.5 (15.156)	3.3/0.5 (15.15)	3.3/0.7(21.21)
TLB 520	3.2/0.6 (18.75)	3.4/0.3 (8.82)	3.4/0.7(20.58)
TLB 521	3.4/0.6 (17.64)	3.4/0.7(20.58)	3.3/0.7(21.21)
TLB 543	3.5/0.6 (17.14)	3.2/0.4 (12.5)	3.4/0.7(20.58)
TLB 544	3.3/0.7 (21.21)	3.3/0.7 (21.21)	3.4/0.7(20.58)
TLB 554	3.4/0.3 (20.58)	3.2/0.7(21.87)	3.4/0.6(17.64)
TLB 580	3.5/0..7 (20)	3.5/0.8(22.8)	3.5/0.7(20)
TLB 582	3.5/0.7 (20)	3.3/0.8 (24.2)	3.2/0.8(25)
TLB 590	3.5/0.7 (20)	3.4/0.7 (20.5)	3.5/(22.85)
*Positive control	3.4/0.9 (26.4)	3.3/0.9 (27.27)	3.4/1(34)
**Negative control	0	0	0

*, Triton 100x **, PBS.

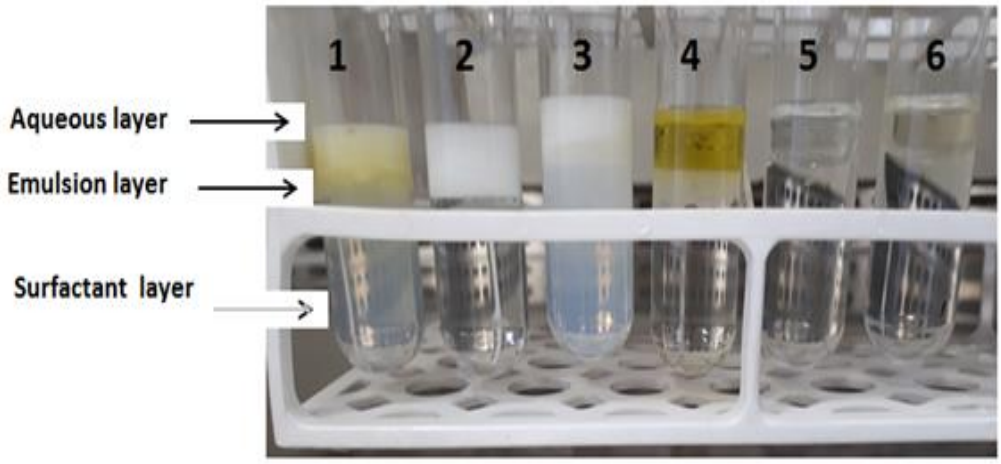

Figure 15. Emulsification index (EI) determination for biosurfactant production of selected *Lactobacillus* strains. 1, 2, and 3, emulsification index of olive oil, sunflower oil and kerosene oil, respectively, mixed with triton100X (0.1) as chemical biosurfactant (positive control); 4, 5, and 6 emulsification indexes of TLB 590 extracted biosurfactant mixed with olive, sunflower and kerosene separately.

6.13. Antimicrobial Assessment of Crude Biosurfactants Isolated from Selected *Lactobacillus*

A partial inhibition rates were observed of the selected six strains against the *Y. pseudotuberculosis* ATCC 911 and *S. pyogenes* ATCC 12344 under biosurfactant concentration of 25 mg/mL, which ranged from (58 to 92 %) while for the *B. cereus* RSKK 709 Roma showed moderate inhibition ranging from 42 to 49 % under 25 mg mL^{-1} concentration. The other concentrations, 12.5 and 6.25 mg mL^{-1} showed insignificant growth inhibition rates (Figures 16, 17 and 18).

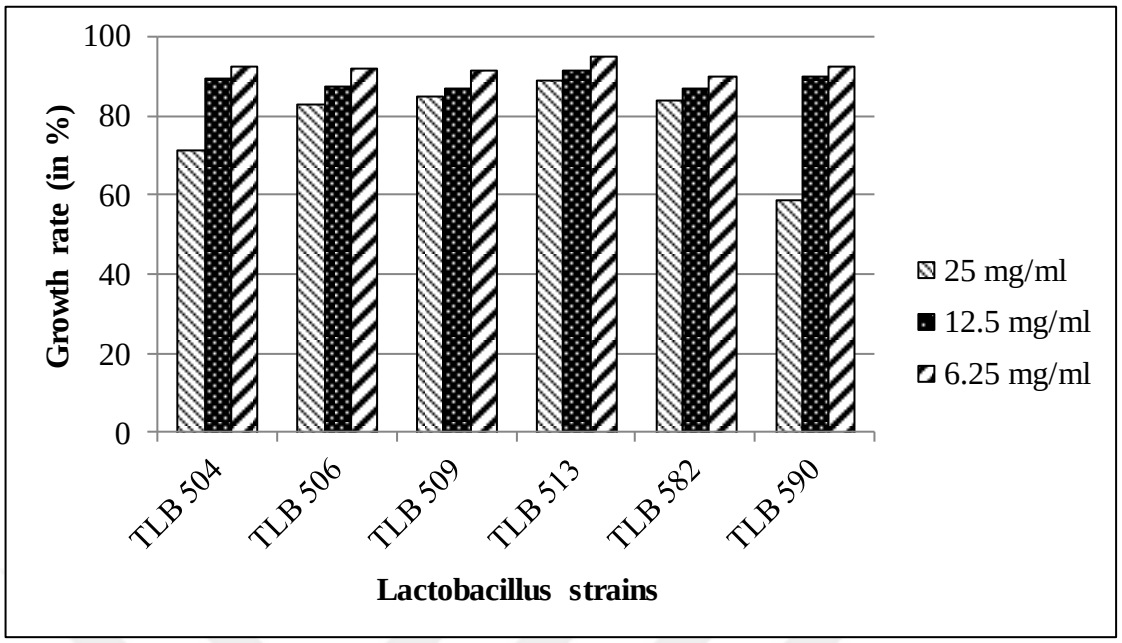

Figure 16. Antimicrobial assessment of crude biosurfactants isolated from six strains of *Lactobacillus* against *Y. pseudotuberculosis* ATCC 911.

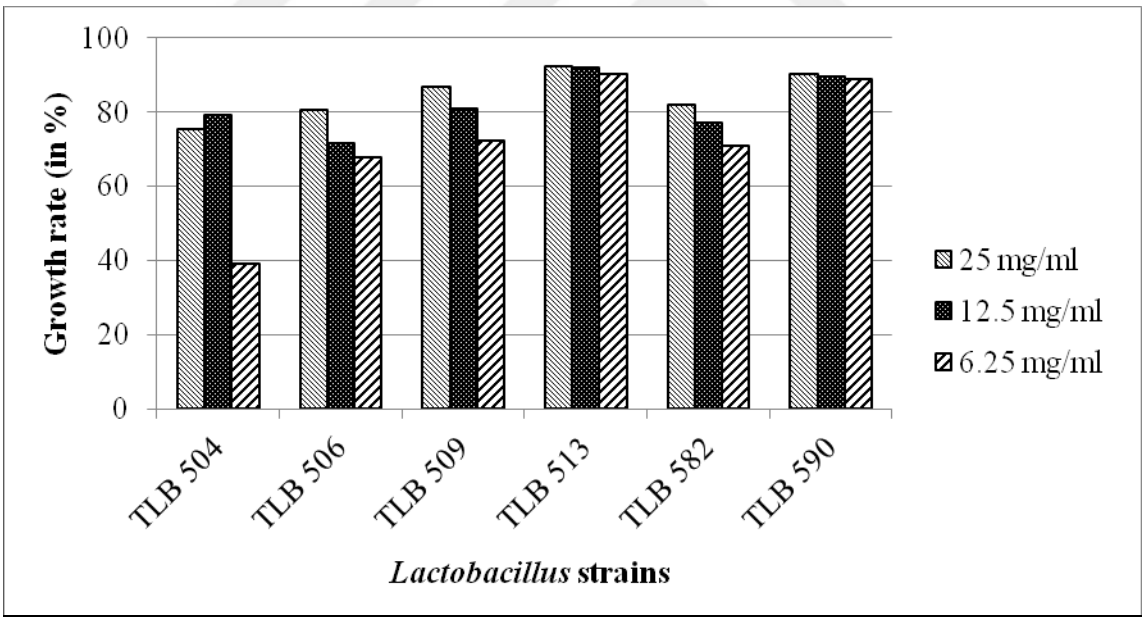

Figure 17. Antimicrobial assessment of crude biosurfactants isolated from six strain of *Lactobacillus* against *S. pyogenes* ATCC 12344.

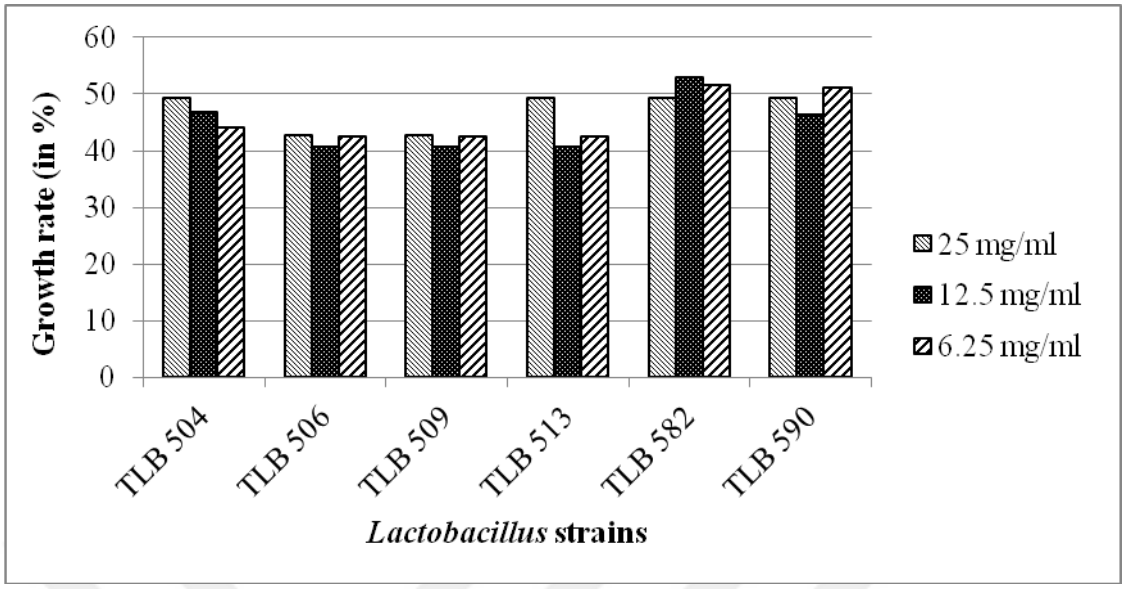

Figure 18. Antimicrobial assessment of crude biosurfactants isolated from six strain of *Lactobacillus a*gainst *B. cereus RSKK* 709 Roma.

6.14. Molecular identification of selected *Lactobacillus* strains

The 16S rDNA gene region of about 1.5 kb was amplified and each fragment gel purified for sequencing (Figure 19). Of 14 strains, 16S rDNA gene regions of 10 strains were successfully sequenced. The sequence data were compared with published data in GenBank (National Centre for Biotechnology Information, USA). The sequence data revealed that all 10 strains were higly homologous to *Lactobacillus plantarum* subsp. *Plantarum* (Table 19).

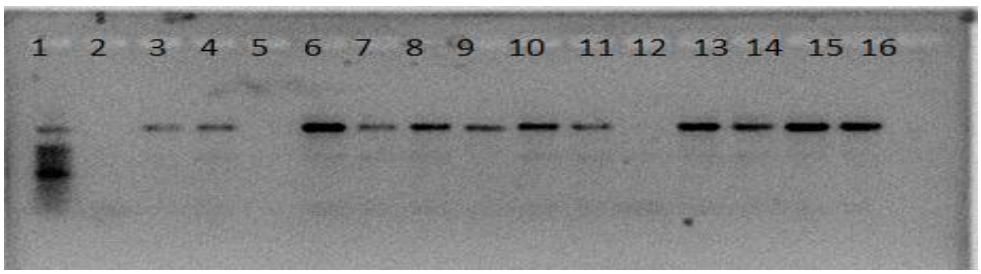

Figure 19. Agarose gel electrophoresis of 1.5 kb 16S rDNA regions of *Lactobacillus* strains by PCR.1, MW marker; 2, negative control; 3, TL 504; 4, TLB 506, 5, TLB 509; 6, TLB 513; 7, TLB 514; 8, TLB 519; 9, TLB 520; 10, TLB 521;11, TLB 543;12, TLB 544;13, TLB 554;14, TLB 580; 15, TLB 582; 16, TLB 590.

6.15. Identification of Lactobacillus by Matrix Assisted Laser Desorption/ Ionization Time of Flight Mass technique.

Identification of all 14 strains were carried out using the MALDI-TOF. With this technique, all 14 strains were identified as *L. plantarum,* to the species level. Comparison of 16S rDNA sequence-based identification of 10 strains and MALDI-TOF-aided identification of 14 strains are presented in Table 19.

Table 19. Comparison of genetic identification and MALDI-TOF methods for identification of selected *Lactobacillus* strains.

Strains	Sequence-based Identification	% identity /compared bp	MALDI-TOF identification
TLB 504	*L. plantarum subsp. plantarum*	100 / 1412	*L. plantarum*
TLB 506	*L. pentosus* *L. plantarum* subsp. *argentoratensis* *L. paraplantarum* *L. plantarum* subsp. *plantarum*	100 / 568	*L.plantarum*
TLB 509	*L. plantarum* sbusp. *plantarum*	99.86 / 1409	*L. plantarum*
TLB 519	*L. plantarum* subsp. *plantarum*	100 / 758	*L.plantarum*
TLB 521	*L. herbarum* *L. pentosus* *L. fabifermentans* *L. plantarum* subsp. *aergentoratensis* *L. paraplantarum* *L. plantarum* subsp. *plantarum*	100 / 336	*L. plantarum*
TLB 543	*L. pentosus* *L. paraplantarum* *L. plantarum* subsp. *plantarum*	100 / 109	*L. plantarum*
TLB 582	*L. herbarum* *L. pentosus* *L. fabifermentans* *L. plantarum* subsp. *argentoratensis* *L. paraplantarum* *L. plantarum* subsp. *plantarum*	100 / 306	*L. plantarum*
TLB 590	*L. plantarum* subsp. *plantarum* *L. pentosus* *L. plantarum* subsp. *argentoratensis* *L. paraplantarum*	100 / 729	*L. plantarum*

7. DISCUSSION

Investigating the presence of microorganisms in certain foods and precise identification of the strains are of great importance in their potential industrial applications as biopreservatives and probiotics (3). Therefore, the aims of this study were to isolate lactobacilli from different fermented products, both commercial and homemade products produced by the locals and sold in the city of Trabzon, and identify and investigate their features for probiotic potentials.

In the study, a total of 103 of presumptive *Lactobacillus* colonies were recovered from 50 different fermented products. Most *Lactobacilli* were isolated from homemade white cheese samples, which account for 46 (44.6%) isolates and followed by the commercial yogurt samples, which account for 23 (22.3%) out of 103 strains. Only 2 (1.94%) strains were obtained from pickles and turnip juice samples.

Primary identifications of the isolates were done based on their Gram staining reactions and cell morphology examination by light microscopy, catalase, oxidase tests and their ability to ferment different sugars, which is commonly used to identify the species belong to the genus *Lactobacillus* (97). Biochemical test results revealed that *Lactobacillus* isolates were negative for catalase and oxidase tests, and they were able to utilize most of the sugars as carbon source.

Among the fermented food products, fermented milk was regarded as the major source of probiotic bacteria while cheese was less frequently considered as source for probiotic strains, we recover more strains from homemade white cheese than yogurt in our study. To select the potential candidates with probiotic features, all 103 isolates were analyzed by their biochemical and physiological properties. First, all isolated were tested for their resistance to stomach acidity and high bile concentration. Based on these criteria, 29 strains out of 103 were able to resist and grow in MRS broth with pH ranging from 6.5 to 2.0. Among these, five strains, designated as TLB 519, TLB 582, TLB 520, TLB 554, and TLB 590 were showed high resistant to acidity as displayed in Table 5. Another criterium for selection of the potential probiotic bacteria, tolerance to bile salt was also carried out with 29 strains. Among them, fourteen strains were found to resist the bile salt concentration of 0.3% for 3 to 6 h of incubation. Among them, TLB 513, TLB 520, TLB 521, TLB 543, TLB 544, and TLB 554 showed the most

resistance (Table 6 and Figure 6). Similar results were reported in a previous study (98) which reported that *L. casi* isolated from kooze cheese had the ability to resist and grow under acid and bile salts concentration. The probiotic bacteria must resist to low pH or high concentration of bile salts for at least 90 min under in the digestive system before colonization in the gastrointestinal cell, withstand the harsh conditions of the host before displaying health-promoting effects (99,100).

In the present study, 14 *Lactobacillus* having acid and bile salt tolerance were selected for further characterization. To test their viability rate, gastric pepsin and intestinal pancreatin at 0.3% and 0.1% concentration, respectively, were used. The probiotic activities of the strains were evaluated by observing their surviving rates which were indicated by calculating the CFU at time zero ($t_{0\ h}$) and after 3 h of incubation in the presence of pepsin and 4 h in the presence of pancreatine incubation. From 14 *Lactobacillus* strains, TLB580, TLB506, and TLB514 showed surviving rates of 86 %, 79.4 %, and 70%, respectively in the presence of pepsin, and 97.7%, 96.0%, and 90 %, respectively, in the presence of pancreatin.

Based on the results from pepsin and pancreatin resistance tests, 14 isolates were able to resist the acidity of the stomach and high concentration of bile salt that could enable them to pass through the stomach and reach to the intestinal systems. These features may qualify them as potential candidates for being probiotic strains. Our findings are comparable with the results of a previous study (101) in which *L. plantarum* and *L. brevis* species were found to have similar desirable features.

In regards to safety of the probiotic bacteria, some of the 14 *Lactobacillus* strains, TLB 519, TLB 520, TLB 521, TLB 543, and TLB 544 showed alpha haemolysis. Since potential probiotic bacteria should never lyse human red blood cells, these five strains were excluded from the study. The remaining nine nonhaemolytic strains were further characterized for their potential probiotic features.

Overuse or misuse of antibiotics resulted in the increase of antibiotic resistance worldwide in recent years. Antibiotic resistance gene transfer among bacteria, including probiotic community should be considered during selection of probiotic strains. Therefore, the sensitivity of probiotic bacteria to the widely used clinical antibiotics and

the presence of antibiotic resistance determinants in their genome must be screened (102).

Although sensitivity to antibiotics for bacteria is a desirable feature, the beneficial bacteria like lactobacilli must be antibiotic tolerant and stay viable and stable inside the human body. However, live microorganism that are used in feed as active supplement should not add additional antimicrobial resistance genes to the gut microbiota which is very rich in bacterial communities with resistance genes to further increase the risk of transfer of drug resistance (103).

Although it is reasonable to assume that gene transfer from viable microorganisms to other microorganisms will occur in an open environment such as the gastrointestinal tract, intrinsic resistance is presumed to present a minimal potential risk for horizontal transfer, while acquired resistance mediated by added genes in regard to having a high potential for lateral transfer (104).

The antibiotic susceptibilities of 14 strains were evaluated using most clinically used antibiotics (Table 10). All 14 isolates were found to be sensitive to amoxicillin, azithromycin, chloramphenicol, erythromycin, penicillin, and streptomycin, and they were resistant to vancomycin, ciprofloxacin, and teicoplanin. These results were similar with the results of a previous study (105), in which many of the *Lactobacillus* strain isolated from probiotic products were sensitive to the most widely used antibiotics, and lactobacilli are naturally resistant to some of these antibiotics that used in this study (106). The sensitivity of these strains to commonly used antibiotics may be explained by the limited use of these antibiotics, if any, by the local farmers in the region as feed supplements.

The selection criteria of typical probiotic strains are complex and involved many factors. One of the other important criteria for selection of the probiotic microorganisms is the adhesion capacities on the intestinal cells. It was documented that adhered probiotic strains could interact with immunomodulatory cells of the intestinal mucosa like leucocytes which in turn facilitate the phagocytosis process against different pathogens (41).

In our study, the ability of *Lactobacillus* strains for adhering to HT-29 cells was evaluated by two methods; the first one was measuring indirectly the number of the

adherent bacteria after 3 h of incubation with HT-29 cells represented by colony count after plating the adhered bacteria on MRS agar. Based on the colony counts, six strains, TLB 509, TLB 506, TLB 504, TLB 513, TLB 519, and TLB 590 had the highest adherence ability to H2-29 cells, with 79.23 %, 78.7 %, 78.3 %, 74.1%, 73.18 %, and 73.01% adherence ability, respectively. These six strains were also evaluated for their adherence ability to H2-29 cells using light microscope after staining the cells with crystal violet. Based on the results of the staining method, the adherence ability of bacteria was categorized into three levels, which include non-adhesive, adhesive and strongly adhesive. Our findings indicated that six strains TLB 590, TLB 506, TLB 513, TL 509, TLB 519, and TLB 504 exhibited adhesive score of 102±1.4, 98±1.5, 96±1.84, 93±1.35, 93±1.15, and 88±1.2, respectively. The TLB 590 showed a strong adhesive score among the other strains. Similar finding of adhering properties was reported in one previous study (107).

The inhibitory actions of the lactobacilli were tested from the supernatants of each of 14 cultures for the presence or absence of antimicrobial substances due to organic acids and/or H_2O_2. The antimicrobial activity of each supernatant subjected to neutralization for acids and catalase treatment to inhibit H_2O_2 was evaluated against a number of common intestinal pathogenic bacteria and *C. albicans*. The results of agar-well diffusion analysis showed that the materials released by 14 *Lactobacillus* strains were mostly inhibitory to *E. coli* ATCC 29212, *E. faecalis* ATCC 29212 and *S. pyogenes* ATCC 12344 due to the activity of H_2O_2, organic acids or both. The antibacterial activities were diminished upon neutralization and treatment of the supernatants with catalase. *C. albicans* ATCC 10231, *P. mirabilis* ATCC 13315, *S. typhi* ATCC 14028, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, and *K. pneumonia* ATCC 13883 were not inhibited by any of the supernatants tested. Inhibition due to bacteriocin activity was not found when trypsin treated supernatants used.

In industrial fields, lactic acid bacteria occupy a very important place among microbes due to their large use in different food fermentation processes. They added flavor, texture and nutrition value features for the fermented products (108). With increase resistance of antibiotic among most bacteria, an alternative way of replacing antibiotic was focused on using probiotic bacteria and their antimicrobial compounds for prevention of infections (109). The most popular antimicrobial compounds produced

by *Lactobacillus* include bacteriocins, organic acid, and hydrogen peroxide.

Bacterial strains can harbor temperate or prophages in their genomes which may lead to the lysis of the starter bacterial cultures during food fermentation, lead to delay or cause the complete failure which leads to losing of the product. Contamination of fermented foods with phages economically regarded as the most serious cause of fermentation failure (108). In the present study, 14 *Lactobacillus* strains that were selected as potential probiotic candidates, were also screened for the presence of prophages in their genomes by induction method. There were no lytic phages detected in the genomes of the strains, although prophages have been isolated and characterized one of the previous studies from fermented milk products (79).

Among the other antimicrobial agents that lactobacilli synthesize, biosurfactant synthesis by the selected strains of lactobacilli were also investigated. Biosurfactants are surface-active compounds that are produced by different pathogenic and nonpathogenic bacteria like *P. aeruginosa* and *Lactobacillus* spp. Biosurfactants produced by lactobacilli could have essential applications in the food industry as antimicrobial agents (91).

The six strains that were selected for good adhesive features were also evaluated for their biosurfactant production ability using three different methods; oil spreading test, drops collapse assay and emulsification index (EI). The results of the oil spreading test indicated that TLB 504, TLB 506, TLB 509, TLB 513, TLB 582, and TLB 590 had high scores for oil displacement especially when mixed with kerosene oil as compared to sunflower and olive oils (Table 16). For the drops collapse assay, TLB 504, TLB 506, TLB 509, TLB 513, TLB 582, and TLB 590 strains had higher values (Table 17). The finding of the oil spreading together with drop collapse assay confirmed that the selected strains were regarded as a producer for cell-bound biosurfactants. Our findings were similar to previous two reports (110, 91).

For more confirmation, the capacity of crude biosurfactant to emulsification was evaluated against three types of oils: kerosene, sunflower and olive oil as water-immiscible substrates in comparison with results of positive and negative controls. The results showed that the highest (IE) values were found with olive and sunflower oil treatments while the lowest (IE) values were observed with kerosene treatment for all

Lactobacillus strains under the investigation of this study (Table 18). Our results agree with a previous report (91), which observed that the isolated *Lactobacillus* from Romanian fermented traditional food products *L. plantarum* L26, *L. plantarum* L35, and *L. brevis* L61 were good biosurfactant producers. In the literature, the antimicrobial and anti-adhesive characteristics of biosurfactant against different pathogens such as *C. albicans, E. coli, B. cereus, S. aureus, P. aeruginosa, S. agalactia,* and *S. epidermidis* were also reported (91, 92).

In our study, antimicrobial activities of crude cell-bound biosurfactants were observed against *Y. pseudotuberculosis* ATCC 911 and *S. pyogenes* ATCC 12344 in the presence of 25 mg mL^{-1} biosurfactant concentrations ranging from 58% to 92% while for the *B. cereus* ATCC 709 showed moderate inhibition rate ranging from 42% to 49% with same biosurfactant concentration (Figures 16-18). The other concentrations, 12.5 and 6.25 mg mL^{-1} showed constant growth inhibition rate. Our data were similar to the data presented in a previous study (92), which documented that a total growth inhibition of *E. coli, S. agalactiae* and *S. pyogenes* with a biosurfactant in the presence of 25 mg mL^{-1}.

Although the best biosurfactant producer microorganisms were *Bacillus* and *Pseudomonas* spp, *Lactobacillus* strains are generally recognized as safe (GRAS) microorganisms and they are preferred bacteria in various food products.

Primary identification of 14 potential probiotic lactobacilli was done based on the Bergey's Manual of Determinative of Bacteriology (110). All the strains were able to grow at 15 C, but not at or above 45 C, in the presence of at 1 to 7% NaCl. Morphological features, Gram staining features and carbohydrate utilization patterns were also used for primary identification.

For more accurate identification of 14 lactobacilli, MALDI-TOF and molecular identification methods were employed. The MALDI-TOF-based identification revaled that all strains belong to *Lactobacillus plantarum species.* The 16S rDNA sequence analysis revealed that 10 of 14 strains, TLB 504, TLB 506, TLB 509, TLB 513, TLB 514, TLB 519, TLB 521, TLB 543, TLB 582, and TLB 590 belong to *Lactobacillus paraplantarum* and *Lactobacillus plantarum subsp. plantarum* (Table 19). Both methods, MALDI-TOF- and 16S rDNA sequence analysis-based identifications of

lactobacilli at the species level in agreement with each other, the 16S rDNA sequence analysis provide identity at the subspecies level.

8. CONCLUSIONS AND RECOMMENDATIONS

1. In the current study, 103 *Lactobacillus* strains were isolated from different fermented dairy products. Only 29 strains were able to grow under different pH concentrations which are the first step to screen other probiotic properties.
2. Fourteen out of 29 strains showed good to excellent tolerance to acids and bile salts. From these, *Lactobacillus* strains TLB 506, TLB 514, TLB 580 have shown to have strong acid and bile salt tolerance.
3. All 14 *Lactobacillus* strains tested for their susceptibility to ten commonly prescribed antibiotics. All isolates presented sensitivity to amoxicillin, azithromycin, chloramphenicol, erythromycin, gentamycine, penicillin, and streptomycin, and they showed intrinsic resistance to ciprofloxacin, teicoplanin, and vancomycin.
4. Analysis of the adherence ability of *Lactobacillus* stains on human HT-29 cells indicated that six strains including TLB 504, TLB 506, TLB 509, TLB 513, TLB 519, and TLB 590 showed high adhesion index.
5. The 14 selected *Lactobacillus* were investigated for their antimicrobial metabolite production ability by agar well-diffusion method, and all strains were confirmed for their organic acids and hydrogen peroxide production indicated by the inhibition zone against certain pathogens.
6. Induction of all 14 strains for the presence of prophage and bacteriocin production did not yield any lytic phage or detectable bacteriocin.
7. Primary screening of for the biosurfactants was done for each strain by modified drop collapse method, oil spreading test, and emulsification index, and six strains including TLB 504, TLB 506, TLB 509, TLB 513, TLB 582, and TLB 590 were able to produce extracellular biosurfactants.
8. Overall, nine out of 14 strains displayed probiotic features.
9. Further in vitro and in vivo investigation are required to be undertaken to assess the probiotic features in the complex biological environments. Therefore, the following recommendation is forwarded as a future direction.
10. Investigation on the interactions of the currently isolated *Lactobacillus* strains with indigenous organisms of the host and an in vivo model.
11. Investigating the different environmental and physiological stress on the functionality of the strains by comparing with known commercial strains.

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10. ETHICAL COUNCIL APPROVAL

T.C.
KARADENİZ TEKNİK ÜNİVERSİTESİ REKTÖRLÜĞÜ
KTÜ TIP FAKÜLTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURUL
BAŞKANLIĞI

Sayı : 24237859-283
Konu: Etik Kurul onay belgesi

02/05/2017

Sayın; Prof. Dr. Faruk AYDIN
Tıbbi Mikrobiyoloji ABD.

"Farklı Gıdalardan İzole Edilen Laktobasil Türlerinin Probiyotik ve Biyosürfektan Özelliklerinin Araştırılması" başlıklı etik kurul 2017/75 protokol numaralı tez çalışması raportör ve etik kurul görüşleri doğrultusunda; tıbbi etik açıdan uygun olduğuna karar verilmiştir.

Bilginizi ve gereğini rica ederim.

Prof.Dr.Faruk AYDIN
Etik kurul Başkanı

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KTÜ TIP FAKÜLTESİ BİLİMSEL ARAŞTIRMALAR
ETİK KURULU KARAR FORMU

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	"Farklı Gıdalardan İzole Edilen Laktobasil Türlerinin Probiyotik ve Biyosürfektan Özelliklerinin Araştırılması"
	ARAŞTIRMANIN PROTOKOL/PLAN KODU	2017/ 75
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI UNVANI/ADI/SOYADI	Prof. Dr. Faruk AYDIN
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ UZMANLIK ALANI	Tıbbi Mikrobiyoloji
	TEZ SAHİBİ/DİĞER ARAŞTIRICILAR. ÜNVANI/ADI/SOYADI	Doktora Öğr.Jaleel SAMANJE, Prof.Dr.Ali Osman KILIÇ, Prof.Dr.Osman Birol ÖZGÜMÜŞ
	DESTEKLEYİCİ	
	ARAŞTIRMANIN NİTELİĞİ	
	ARAŞTIRMANIN TÜRÜ	TEZ ☒ AKADEMİK AMAÇLI ☐
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ☒ / ÇOK MERKEZLİ ☐ / ULUSAL ☐

	Belge Adı	Tarihi	Versiyon Numarası	Dili
DEĞERLENDİRİLEN BELGELER	ARAŞTIRMA PROTOKOLÜ/PLANI			Türkçe ☒ İngilizce ☐ Diğer ☐
	BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU			Türkçe ☒ İngilizce ☐ Diğer ☐
	OLGU RAPOR FORMU			Türkçe ☒ İngilizce ☐ Diğer ☐

DEĞERLENDİRİLEN DİĞER BELGELER	Belge Adı		Açıklama
	TÜRKÇE ETİKET ÖRNEĞİ	☐	
	SİGORTA	☐	
	ARAŞTIRMA BÜTÇESİ	☐	
	BİYOLOJİK MATERYEL TRANSFER FORMU	☐	
	İLAN	☐	
	YILLIK BİLDİRİM	☐	
	SONUÇ RAPORU	☐	
	GÜVENLİLİK BİLDİRİMLERİ	☐	
	DİĞER:	☐	

Sayfa 1

KTÜ TIP FAKÜLTESİ BİLİMSEL ARAŞTIRMALAR
ETİK KURULU KARAR FORMU

KARAR BİLGİLERİ	Karar No: 10	Tarih: 24/04/2017
	Prof.Dr.Faruk AYDIN'ın sorumluluğunda yürütülmesi planlanan Doktora Öğr.Jaleel SAMANJE'ye ait "Farklı Gıdalardan İzole Edilen Laktobasil Türlerinin Probiyotik ve Biyosürfektan Özelliklerinin Araştırılması" başlıklı 2017/75 no.lu ve yukarıda başvuru bilgileri verilen araştırma/tez başvuru dosyası ile ilgili belgeler araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş, gerçekleştirilmesinde etik sakınca bulunmadığına; toplantıya katılan etik kurul üyelerinin oy birliği ile karar verilmiştir.	

KTÜ TIP FAKÜLTESİ BİLİMSEL ARAŞTIRMALAR ETİK KURULU KARAR FORMU	
ÇALIŞMA ESASI	Klinik Araştırmalar Hakkında Yönetmelik, İyi Klinik Uygulamaları Kılavuzu
BAŞKANIN UNVANI / ADI / SOYADI:	Prof.Dr.Faruk AYDIN

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	Cinsiyet		İlişki *		Katılım **		İmza
Prof.Dr.Faruk AYDIN Başkan:	Tıbbi Mikrobiyoloji	KTÜ Tıp Fakültesi	E ☒	K ☐	E ☒	H ☐	E ☐	H ☐	OYLAMAYA KATILMADI
Prof.Dr.Gamze ÇAN Başkan Yrd.	Halk Sağlığı	KTÜ Tıp Fakültesi	E ☐	K ☒	E ☐	H ☒	E ☐	H ☒	İZİNLİ
Prof.Dr.S.Caner KARAHAN Üye:	Tıbbi Biyokimya	KTÜ Tıp Fakültesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Prof.Dr.S. Murat KESİM Raportör:	Tıbbi Farmakoloji	KTÜ Tıp Fakültesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Prof.Dr.Yılmaz BÜLBÜL Üye:	Göğüs Hastalıkları	KTÜ Tıp Fakültesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Prof.Dr. Murat LİVAOĞLU Üye:	Plastik, Rekons. ve Estetik Cer.	KTÜ Tıp Fakültesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Doç.Dr.Şafak ERSÖZ Üye:	Tıbbi Patoloji	KTÜ Tıp Fakültesi	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Y.Doç.Dr.Demet SAĞLAM AYKUT Üye:	Ruh Sağlığı ve Hastalıkları	KTÜ Tıp Fakültesi	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Prof.Dr.Murat ÇAKIR Üye:	Çocuk Sağlığı ve Hastalıkları	KTÜ Tıp Fakültesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	

* :Araştırma ile İlişki
** :Toplantıda Bulunma

Sayfa 2

11. CURRICULUM VITAE

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Education information

Degree	Name of school	Graduation date
Secondary school	AL-Markaziya / Basrah	2000
B SC	College of Health and Medical Technology/ Baghdad	2006
M Sc	College of Health and Medical Technology/ Baghdad	2012

Languages

1. Arabic
2. English
3. Turkish

Posters

1. Tabbouche S., Tüfekci E.F**., Samanje J**., Abdurrahman M., Reis A., Kiliç A.O., "Callosobruchus maculatus (fasülye böceği) bağırsak florasından izole edilen bakterilerin morfolojik ve genetik karakterizasyonu ve antimikrobiyal duyarlılıklarının saptanması", 36. Türk Mikrobiyoloji Kongresi, Antalya, Türkiye, 12-16 Kasım 2014, ss.243-244.
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3. **J. Samanje1**, O. B. Ozgumus2, F. Aydin1, A. O. Kilic1 "Isolation and Identification of *Lactobacillus* Strains from Different Fermented Foods".10th Balkan Congree of Microbiyology, Sofia, Bulgaria, November 16th-17th, 2017.

Project

- "Investigation of probiotic and biosurfactant properties of *Lactobacillus* isolated from different fermented foods". Supported by the Scientific Research Projects Coordination Unit of Karadeniz Technical University (Project number: TDK-2015-6801)