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# Study of antimicrobial properties of lactic Acid bacteria detected in Yogurt and Cheese on pathogenic bacteria in Ardabil"

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ABSTRACT

Lactose indigestion or intolerance is a common issue since long around the globe. This study involves the determination of lactic acid bacteria role during pathogenic infection. The lactic acid bacteria from dairy products are isolated using MRS media to determine their beneficial and harmful effects on the growth of pathogenic microorganisms. The lactic acid bacteria include lactose fermenters specifically *Lactobacillus spp*. Eight isolates were tested to determine their antimicrobial activity against pathogens. The zone of inhibition ranges from 11-18 mm by *Lactobacillus spp*. (*Lactobacillus casei*, *Lactobacillus salivarius*, *Lactobacillus brevis*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Lactobacillus plantarum*). This gives the susceptibility pattern against the pathogens (*Salmonella typhi*, *Helicobacter pylori*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*). 84% of the isolates show the susceptibility pattern against pathogens. The lactic acid bacteria boost immunity by secreting extracellular antimicrobial products in a competitive hostile environment. The treatment through antibiotics can be alternated by probiotics intake. This will also avoid pathogenic microorganism to become resistant that is an enigma in pharma industries.

## 1. Introduction

Lactose fermenters were first identified by Scheele and curd in 1857 and found that the curd formation was due to microbes (Saranraj et al., 2013). They are commonly recognised as harmless i.e. generally regarded as safe (GRAS microbes) which play a central role in the fermentation to maintain the quality of the food products. Since, fermentation products have mostly organic acids (which have low pH) they do help in the preservation of food products and play an important role in the maintenance of natural microflora (Yang et al., 2012). Lactic acid bacteria (LAB) do not only produce organic acids but also produce other compounds like; carbon dioxide ($CO_2$), hydrogen peroxide ($H_2O_2$), diacetyl (2,3-butanedione), and bacteriocins. LABs are used for the fermentation of foods like; butter, cheese, yogurt, and Kimchi. Lactic acid bacteria which are present in the fermented and raw milk belong to the genera; *Enterococcus, Lactobacillus, Streptococcus, Weissella, Leuconostoc, Oenococcus, Carnobacterium, Pediococcus,* and *Lactococcus* (Federici et al., 2014).

LAB are present in many sources such as soil, intestines of animals, different milk products (fermented dairy products), poultry farms, and fermented foods. They are generally associated with environment enriched with nutrients such as milk, meat, cheese, beverages and vegetables. Also, lactic acid bacteria are present in lakes, soil, and intestinal tracts of

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animals and humans (Tserovska et al., 2002; Ibrahim S., 2016). Mostly *Lactobacillus spp.* (*L. johnsonii, L. reuteri, L. gasseri, L. acidophilus L. rhamnosus, L. casei*), *and genus Bifidobacteria* (*B. animalis subsp. lactis, B. Bifidum, B. longum subsp. Infantis, B. longum subsp. longum*) are included in probiotics LA (Ouwehand et al., 2002; De Angelis M & Gobbetti M., 2016).

Lactic acid bacteria exhibit an ability to produce a range of bacteriocins i.e., antimicrobial proteinaceous compounds. These compounds assist with an antimicrobial property of LAB against a broad range of gram-positive bacteria. Lactic acid bacteria are classified into two groups based on their end product of fermentation, known as either *Homo-fermenters* or *Hetero-fermenters*. The Homo-fermenter produces lactic acid as the major end product of glucose fermentation. While, hetero-fermenter yields lactic acid, carbon dioxide, acetic acid and ethanol, as end products from the fermentation of glucose (Tserovska et al., 2002; Liptáková et al, 2017). LABs are mostly used in the food industry because they are implacable for their characteristic to determine the flavour, nutritional value and texture of fermented products. Lactic acid bacteria have great potential to use in the bio-preservation process because of their safety status.

Cheese and yogurt are produced by the fermentation of the milk to produce desired flavour, acidity, and flavour, under controlled conditions. Fermented yogurt is the prime product from which other products are also made and it acts as probiotics food preservatives (Murry et al., 2004; Shori B., 2013). Lactic Acid Bacteria (LAB) is used as a starter culture in the fermentation of yogurt and cheese. Several factors influence the quality of fermented products such as processing conditions, type of milk, and storage conditions. In all of these, the quality of a starter culture in which LABs act as the most crucial factor which is involved in the development of yogurt with better acidity, flavour, and consistency (Auclair and Accolas, 1983; Vedamuthu E., 2013; Chen et al., 2017). Industrialization of these biological products such as; yogurt and cheese, has enhanced the economic value of lactic acid bacteria.

A term probiotic is coined for the lactic acid bacteria, and they provide many health benefits. Probiotics have many beneficial effects; it prevents the colonization of infectious bacteria, stops the interaction with enteric pathogens, production of antibodies, and lactose digestion in the small intestine. Probiotic microorganisms enhance enzymes production, immune responses and growth of the organism, pathogens inhibition by providing beneficial nutrients used for the killing of uropathogenic microorganisms. Hence, LABs are used for the treatment of urinary tract infections (Manhar et al., 2016). They also protect against pathogens which cause complications such as; lactose intolerance, colon cancer, and also help in reducing allergies. They have anticholesterol or antihypertensive effects. They also exhibit the excellent potential to decrease oxidative stress, to improve nutritional status, reduce inflammatory cytokines, and provide care for depression disorders (Logan and Katzman, 2005; Anandharaj et al., 2015; Dallal et al., 2015; Huang et al., 2016). *S*heep milk exhibit beneficial microbes like *Enterococcus* genus play an important role in proteolysis that help in maturation of cheese (Silva et al., 2013). Bacteriocins (such as nicin) and lactic acid produced from probiotics contained antibacterial activities (Doron and Gorbach, 2006; Muhsin et al., 2015; Vieco-Saiz et al., 2019). Probiotic bacteria resist the colonization of pathogenic bacteria by competing for cell- surface and nutrients with infectious bacteria are also inhibited by some strains of probiotics due to its enzymes production (Psomas et al., 2001). Probiotics provide immunity by activating B-lymphocytes and T- lymphocytes when any foreign particle enters into body B and T- lymphocytes activates by increasing the level of interleukin which produce antibodies for protection against intestinal diseases (Gotcheva et al., 2002, Yousefi et al., 2018). *Lactobacilli* stop the overgrowth of the bacteria by reducing the production of toxic metabolites. It reduces the toxicity of carcinogenic metabolites by changing the activity of the cancerous enzyme. It reduces the urogenital and *Helicobacter pylori* infections by attaching with urinary tract cells adopting the competitive exclusion process and by decreasing the gastric *H. pylori* concentration (Nagpal et al., 2012). *Lactobacillus spp*. is the most useful microbiota of the intestinal region (resistant to all digestive enzymes) have gram-positive like cell wall. In oesophagus inlets and the human gut, LAB controls the microbiota of the intestine

by secreting antimicrobials and thus preventing any harmful bacteria to accumulate in the intestine (Karami et al., 2017). Nisin which is extracted from *Lactobacilli spp*. is a well-known bacteriocin and it is involved in the inhibition of food adulteration and also help to increase the shelf life of dairy products (Bintsis, 2018). The *lactobacillus* strains are tested against the pathogens; *Salmonella typhi* which is an enteric pathogen that causes the GI tract illnesses and typhoid fever by disturbing the immune system. About 20 million people get affected by this fever with approximate 220,000/annum mortality rate (Mathur et al., 2012), *Pseudomonas aeruginosa* causes the cystic fibrosis, chronic disease affecting the humans affecting respiratory tract illnesses (Hoiby et al. 1977; Gellatly & Hancock, 2013), *Helicobacter pylori* causes gastric cancer or ulcer in infection gift (Gemilyan et al., 2019). *Staphylococcus aureus* results in gastric tract illnesses, and bloodstream infection (Thomer et al., 2016). Resistance to several antibiotics such as penicillin, trimethoprim-sulphamethoxazole (TMP-SMX), quinolones, vancomycin, ceftriaxone, tetracycline, norfloxacin, penicillin and ciprofloxin, is increasing in UTI patients (Mitiku, 2017). *Enterococcus, Pediococcus acidilactici, Lactobacillus brevis* and *Pediococcus acidilactici* were isolated in the conducted study. Strains showed prominent antimicrobial activity. The bacteria produce bacteriocin and hydrogen peroxide as a by-product (Alander et al., 1999; Vieco-Saiz et al., 2019). All the isolated bacteria show excellent activity for the killing of urinary tract pathogens but *L. brevis* is one of prominent in them. Probiotics are used for the treatment of urogenital tract infections like bladder cancer and kidney stones (Duncan et al., 2002, Grin et al., 2013; Khan et al., 2013).

Fermented milk produced by *Lactobacillus* strain GG (ATCC 53103) is used to minimize the *E. coli* infection causing diarrhoea than pasteurised yogurt. It also reported that fermented products and fresh live dairy products stop the growth of pathogenic bacteria such as *Salmonella, Enterococcus faecalis* and *E. coli.* Recently probiotics have approved by FDA that *Lactobacillus rhamnosus* used for preventing diseases including diarrhea, intestinal normal flora regulation, compete with cancer-related diseases and immunity improvements. Infections caused by *Salmonella typhimurium* are also treated by *L. rhamnosus* (McKenney et al., 2010).

*Purpose of the study*

In Iran, there are multiple dairy traditional products like cheese and yogurt available as the source of probiotics. But these dairy products (even pasteurized milk), are the sources of many pathogenic bacteria like *S. typhi*, *S. aureus*, *E. coli*, *H. pylori*, *P. aeruginosa* etc. (Talaei et al., 2015; Owusu-Kwarteng et al., 2020; Fusco et al., 2020). Hence, the sole purpose of the study is to determine the antimicrobial activity of *Lactobacilli spp*. isolated from the dairy products against these pathogenic microorganisms and determining their numbers in different dairy products samples, to evaluate whether the presence of these *Lactobacilli* in milk or related fermented milk products can provide safe status to the respective food or not.

## 2. Materials and Methods

This is a cross-sectional and applied study which was conducted to investigate the effectiveness and frequency of *Lactobacillus* against pathogenic strains of bacteria.

### *2.1. Sampling*

A total of 50 samples of (each of 25 g) cheese and (each of 50 g) fresh and traditional packaged yogurt were collected from different localities in Ardabil city of Iran and kept them at 4$^{o}$C to maintain standards of samples.

### *2.2. Enrichment of samples*

15 g of each sample was inoculated with 200 ml of (De Man, Rogosa and Sharpe agar) MRS broth containing 5 µ/ml of nystatin (antifungal) and incubated in anaerobic conditions (5% $CO_2$) at 37 °C for 24 to 72 hours. This will allow only bacterial cultures to grow exponentially in the less competent environment within less time (Prabhurajeshwar & Chandrkanth, 2017).

### *2.3. Isolation and purification*

The 10 µl of serially diluted and enriched each sample were inoculated on separate plates containing MRS agar and incubated at different temperatures 37°C in anaerobic condition (5%

$CO_2$) for 24 to 48 hours. Colonies isolated from MRS agar were transferred to liquid MRS medium for purification. Culture media were centrifuged at 6000 ° C for 15 minutes at 4 ° C. The precipitate was removed under sterile conditions and the supernatant was kept for further examination. The CFU/ml was found for each of the plates by counting the colonies and dilution factor by using the following formula:

$$CFU = \frac{\text{No. of colonies x Dilution factor}}{\text{Vol. ml plated}}$$

Pure isolated colonies were cultured on MRS agar at 33-37 °C for 24-72 hours in an anaerobic jar. By quadrant streaking, the colonies were purified in three rows of successive culturing at a given temperature (Karam et al., 2017).

### *2.4. Staining*

Gram staining was performed to determine the Gram positive or Gram negative character of bacterial cell wall using 24 hours fresh strain (Karami et al., 2017). In LAB genera, genus *Lactobacilli* which consist of the rarely motile, Gram-positive, non-spore-forming are mainly used as probiotics (Abbas & Mahasneh, 2014). Hence, spore staining was done used to determine the nature of cells using malachite green using 3 days incubated old culture. In spore staining, prepared the smear blotting paper was placed on it, Malakite green was poured for 5 minutes and heat meanwhile. Carefully remove the slide using forceps. Remove the blotting paper and cool for 2 minutes. Rinsed with tap water. Safaranine was added at the end for 2 minutes and washed. Observed under 100x lens in the microscope (Cappuccino and Sherman, 2006; Shuhadha et al., 2017).

### *2.5. Biochemical test for identification of Lactic acid bacteria*

By using Bergey's manual, biochemical tests were performed to determine the genus and species level of these purified strains (Bergey's Manual, 2000).

### *2.6. Catalase Test*

Hydrogen peroxide is extremely toxic superoxide produced by microorganisms during aerobic respiration. If substances are enzymatically degraded, then they are accumulated and results in the death of the microorganism. Catalase enzyme produced by microorganisms and rapidly degrades hydrogen peroxide into oxygen radicals and water. In the catalase test, a glass slide was taken and labelled it with the test organisms. Placed three or four drops of 3% hydrogen peroxide on glass slide then picked up a small colony of a fresh culture of bacteria with the help of inoculating loop and mixed it with hydrogen peroxide. The culture must be 24 hours fresh to achieve good results. Immediately bubble formation was observed. The presence of bubble formation indicated that catalase test is positive and if there is no bubble formation then the result will be negative (Pan et al., 2009; Shuhadha et al., 2017).

### *2.7. Growth at different temperatures*

Different temperatures affect the organic acid production in *Lactobacilli spp* (Quin et al., 2012). MRS agar was prepared and poured into the petri plates and isolated strains of *Lactobacillus* were streaked on it. Incubated at different temperatures 15 and 42°C in an anaerobic jar for 24-72 hours.

### *2.8. Sugar fermentation test*

When sugar is fermented by microorganisms, acid products or gas are produced. End products produce after fermentation are butyric acid, acetone, carbon dioxide, lactic acid, ethyl alcohol, formic acid, butyl alcohol, acetone, acetic acid and hydrogen. These acid products reduce the pH and observe as a colour change. Phenol red is mostly used as a pH indicator. Inverted Durham tubes are used for gas production. For this purpose, MRS medium supplemented with 1% different sugar content (separately including lactose, ribose, arabinose, galactose, glucose, gluconate, maltose, melitose, maltose, rhamnose, sorbose, leucine, xylose, fructose, cellobiose, mannitol, sucrose, and mannose) was used. The use of sugar depends on the presence of digestive enzymes present in lactose fermenters. After preparing the media, filled into test tubes and Durham tubes were inserted then autoclaved at $121^0$C for only 3 minutes to prevent the degradation of carbohydrate. Inoculated these test tubes with bacterial culture and incubated for 24 hours.

Observed colour change (De Keersmaecker et al., 2006).

*2.9. Antibiotic susceptibility test*

Antimicrobial susceptibility testing (AST) is a procedure adopted by laboratory technicians. This test is done to check that which antibiotic is effective for the treatment of specific bacteria. The procedure is Muller-Hinton ager (MHA) was prepared and poured into petri plates. Different isolated strains of *lactobacillius* like *L. salivarious, L. delbrueki, L. plantarum, L. bulgaricus, L. acidophilus, L. bulgaricus* and *L. casei* were swabbed on the separate petri plates. Antimicrobial disks Pipracillin (100 µg), Ampicillin (10 µg), amoxyclav (5 µg), ceftazidime (30 µg), chloramphenicol (30 µg), Co-trimoxazole (25 µg), gentamycin (10 µg), tetracycline (30 µg), vancomycin (30 µg), cloxacillin (500 µg), cefotaxime (30 µg), erythromycin (10 µg) and ceftriaxone (30 µg) were dispensed in equal distances using sterile forceps then incubated at 37°C. Results were observed after 24-48 hours (Prabhurajeswar & Chandrkanth, 2017).

*2.10. Pathogenic Strains*

Standard sequenced ATCC bacteria *Salmonella typhi* (ATCC 19214), *Helicobacter pylori* (ATCC 43504), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 10662) are used. The lyophilized strains were provided by Scientific and Industrial Research Center Ardabil, Iran.

*2.11. Antimicrobial activity*
*2.11.1. Disk diffusion method)*

According to CLSI, disc diffusion method was performed using antibiotic discs for determination of resistance to pathogens. Resistant genes are present in the pathogens that have the ability to overcome or tackle the effects of the antibiotics. To determine the resistance pattern in pathogenic strains *Salmonella typhi*, *Helicobacter pylori*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, multiple classes of antibiotics were used. Antibiotics discs Pipracillin, Ampicilline, amoxyclave, ceftazine, chloramphenicol, Co-trimoxazole, gentamycin, tetracycline, vancomycin, gloxacillin, cefotaxin, erythromycin and ceftriaxone were used to check their resistance and susceptibility pattern of isolates. Muller- Hinton Agar was prepared and poured into petri plates then solidify it. Sterile paper disks with a diameter of 6 mm were impregnated with *Lactobacilli* supernatant for 5 minutes and the disks were placed at 37°C for 4 hours to dry completely. The *Lactobacillus* supernatant-impregnated disks were then placed at a certain distance from each other and the edge of the plate on the surface of Müller-Hinton agar medium. Pathogens were swabbed and placed the discs then incubated for 24-48 hours at 37°C.

*2.11.2. Well diffusion method*

In well diffusion method, wells were made with the help of sterilized pasture pipettes to puncture the swabbed MH agar plate with different pathogenic strains *Salmonella typhi*, *Helicobacter pylori*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and inoculums of *Lactobacillus spp*. (0.5 McFarland standards of 100 µl) were poured in each of all wells. The plates were incubated at 37°C. (5% $CO_2$) for 24-48 hours and results were observed. (Germond et al., 2001). The results were analysed using excel for easy understanding of results.

## 3. Results

The sampling method was according to SOPs and store at $4^oC$ for further processing. Almost 50 strains of lactobacillus were isolated for different dairy products and CFU count per ml was calculated. The CFU ranges from 20 to 45 x $10^{-2}$. After isolation and purification, 50 Lactobacillus spp. were isolated from 50 dairy products (25 yogurts and 25 cheese samples). The frequency of isolated and purified strains was determined and described as in Fig 1. Upon microscopy, the cells at 100X look like rod-shaped, purple and are isolated cells. Catalase test was performed and all the isolated strains exhibit, the character of no hydrogen peroxide conversion to hydrogen and oxygen, catalase-negative properties. These isolates were able to ferment sugar (Table 1) and grow at the desired temperatures and according to biochemical confirmatory tests (based on Bergey's manual of systematic bacteriology) belonging to 7 Lactobacillus strains, were including

*Lactobacillus casei, Lactobacillus salivarius, Lactobacillus brevis, Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Lactobacillus plantarum*.

The antimicrobial activity of *Lactobacillus* strains was observed against pathogens. The supernatant of lyophilized strain was used for further culturing and purification if the isolated colonies. The antimicrobial activity zones were ranged between 11-18 mm in diameter, against different pathogenic strains (*Salmonella typhi, Helicobacter pylori, Staphylococcus aureus,* and *Pseudomonas aeruginosa*). The antimicrobial zones were better observed in disc diffusion method rather of well diffusion.

### *3.1. Growth temperature*

Each *Lactobacilli* isolates were tested to assess optimum temperature for the growth of bacteria since temperature impacts organic acid production in them and results are put in Table – 2.

*L. casei*, *L. plantarum* showed growth between 15□C – 30 □C. *L. acidophilus and L. delbrueki* showed optimum growth at the temperature of 45 □C. While other strains like; *L. bulgaricus,* and *L. salivarius* showed growth between the ranges of 30 □C – 45 □C.

### *3.2. Antibiotic susceptibility test*

Different antibiotics were tested against all four pathogenic bacteria. Results were put in three categories as resistant (R), intermediate (I), and susceptible (S) as per the inhibitory effects of each antibiotic on the pathogenic strains. All strains were resistance, intermediate and susceptible to antibiotics as mentioned below in Table 3.

### *3.3. Well Diffusion method*

In well diffusion method, the maximum inhibitory effect was shown by *Lactobacillus casei* against *S. aureus* with 16.35 mm inhibition and the minimum inhibitory effect belonged to *Lactobacillus casei* against *Helicobacter pylori* with 11.10 mm zone of inhibition.

### *3.4. Disk diffusion method*

In disk-diffusion method, the maximum inhibitory effect was observed for the *L. casei* with 17.74 mm zone of inhibition against *H. pylori* and minimum zone of inhibition (11.70 mm) was observed for *L. casei* against *S. typhi*. Rest of the *Lactobacilli spp*. showed good inhibitory actions against all pathogenic bacteria.

Both of these methods' results showed that the antimicrobial compounds were better diffused in disc form rather in well form. *Lactobacillus casei* with the highest inhibition mean of 17.74 mm in the well-diffusion agar method against *H. pylori* and 16.35 mm in the disk diffusion method against *S. aureus*.

By comparing average inhibitory action of *Lactobacilli spp*. for both methods, it was observed that the maximum average inhibition was shown by *Lactobacillus salivarius* (15.10) against all pathogenic microorganisms in the disk diffusion method and while *L. plantarum* showed maximum inhibition of 13.99 mm in the well-diffusion method. The lowest inhibitory activity was shown by *Lactobacillus acidophilus* against all pathogenic microorganisms in both well-diffusion and disk-diffusion methods. *Lactobacillus plantarum* also showed a high ability to inhibit pathogens with an average inhibition of 14 to 15 mm.

With the help of antibiotic susceptibility testing, pathogenic microorganisms were tested against different antibiotics. Most of the pathogenic strains showed resistance or intermediate effect against the antibiotics which were used in the test. Only *S. aureus* showed susceptibility for the amoxyclav and tetracycline While *P. aeruginosa* showed susceptibility against chloramphenicol and tetracycline. But almost all the pathogenic strains showed susceptibility against all of the *Lactobacilli spp* which were isolated from fermented dairy products, proving the potential probiotic and antimicrobial effects of *Lactobacilli*.

## 4. Discussion

*Lactobacillus* seems to be effective against pathogens, because of the secretion of antimicrobials in the environment. These antimicrobials help them to compete for their survival and improve the immunity of their host. The probiotics are the most efficient one to remove the disease-causing organisms from the

environment by suppressing their growth (Capri et al. 2008; Hemrajata & Versalovic, 2013). The *Lactobacilli* strains seem to be an efficient way to improve body function by reducing the harmful microbes through the surroundings. Since they are resistant towards acid, bile, and salts of the stomach they can only grow in that environment. They also produce acids by fermentation and cause the stomach pH to reach low i.e. they ultimately help to reduce the chance of accumulation of any unusual bacteria to live there (Kang et al., 2005; Hemrajata & Versalovic, 2013). The bactericidal effect of *Lactobacilli* was also found against *Salmonella* by reducing the pH of the media in the surrounding. Rezaee et al. (2019) study has found that the antimicrobial activity of LAB against *Helicobacter spp*. LAB can inhibit the activity of its urease, and decrease the adhesion of *H. pylori* bacteria to cell line. It helps in the prevention of *H. pylori* colonization in GI (Doron et al., 2006, Rezaee et al., 2019).

Many studies have also found that *Lactobacilli* show antimicrobial property against the *Staphylococcus aureus*. *L. salivarius* and *L. fermentum* showed inhibitory actions against S. aureus. *L. salivarius* has shown secretory proteins' activity against *S. aureus* and these proteins act as anti-staphylococcal agents by preventing its biofilm (Kang et al., 2017). In another study, *Lactobacillus plantarum* showed antimicrobial property against Methicillin-Resistant *S. aureus* and *P. aeruginosa* (Layus et al., 2020). The probiotics have useful beneficial effects than causing any harm. The study conducted by Tserovska et al. (2002) has proved beneficial effects of LAB in the large scale production of dairy products and inhibit spoilage due to their acid production activities. Goat milk was used to produce cheese and then noticed the presence of faecal coliforms, *enterobacteriacae* and *L. plantarum*. Homemade white cheese contained the beneficial microbial flora like; *Lactobacillus brevis* as the main microorganism (Gotcheva et al., 2002).

**Table 1**. Biochemical sugar fermentation test results

| Strains | Sucrose | Maltose | lactose | Raffinose | Rhamnose | Mannose | Mannitol | Fructose | Sorbitol | Galactose | Xylose | Arabinose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***L. salivarious*** | + | + | + | + | - | + | + | + | + | + | - | - |
| ***L. delbrueki*** | - | - | - | - | + | + | - | + | - | + | - | + |
| ***L. plantarum*** | + | + | + | + | - | + | + | + | + | + | + | + |
| ***L. bulgaricus*** | + | + | + | + | - | + | +/- | + | + | + | + | + |
| ***L.acidophilus*** | + | + | + | + | + | - | + | - | + | - | + | - |
| ***L. brevis*** | - | + | - | + | - | + | +/- | + | + | - | - | - |
| ***L. casei*** | + | + | + | - | - | + | + | + | + | - | - | + |

**Table 2**. Temperature for the growth of *Lactobacilli spp*.

| Sr. No. | *Lactobacilli spp*. | Optimum temperature for growth | | |
|---|---|---|---|---|
| | | **15** °C | **30** °C | **45** °C |
| **1** | *L. casei* | + | + | – |
| **2** | *L. plantarum* | + | + | – |
| **3** | *L. acidophilus* | – | – | + |
| **4** | *L. bulgaricus* | – | + | + |
| **5** | *L. salivarius* | – | + | + |
| **6** | *L. delbrueki* | – | – | + |
| **7** | *L. brevis* | + | + | - |

**Table 3.** Antibiotic susceptibility test of all pathogenic microorganisms.

| **Antibiotics** | ***S. typhi*** | ***H. pylori*** | ***S. aureus*** | ***P. aeruginosa*** |
|---|---|---|---|---|
| Pipracillin | R | R | I | R |
| Ampicilline | R | R | R | R |
| Amoxyclave | R | R | S | R |
| Ceftazidim | R | R | I | R |
| Chloramphenicol | I | I | R | S |
| Co-trimoxazole | R | R | I | R |
| Gentamycin | R | R | R | R |
| Tetracycline | I | I | S | S |
| Vancomycin | R | R | R | R |
| Gloxacillin | R | R | I | R |
| Cefotaxim | R | R | I | R |
| Erythromycin | R | R | R | R |
| Ceftriaxone | R | R | I | R |
| Clindamycin | I | I | R | R |

**Table 4**. Antimicrobial activity of *Lactobacilli* against pathogenic bacteria (*Well-diffusion method*)

| **Pathogenic bacteria** / ***Lactobacilli* spp.** | **Zone of inhibition (in mm)** | | | |
|---|---|---|---|---|
| | ***S. typhi*** | ***S. aureus*** | ***H. pylori*** | ***P. aeruginosa*** |
| ***L. casei*** | 14.10 | 16.35 | 11.10 | 13.42 |
| ***L. plantarum*** | 11.45 | 14.50 | 14.90 | 15.10 |
| ***L. acidophilus*** | 12.80 | 14.70 | 13.20 | 13.10 |
| ***L. bulgaricus*** | 11.50 | 14.40 | 14.10 | 13.84 |
| ***L. salivarious*** | 11.72 | 13.90 | 14.20 | 13.70 |
| ***L. delbrueki*** | 12.50 | 14.70 | 13.80 | 14.40 |
| ***L. brevis*** | 12.72 | 13.90 | 14.20 | 13.50 |

**Table 5.** Antimicrobial activity of *Lactobacilli* against pathogens (Disk-diffusion method)

| **Pathogenic bacteria** / ***Lactobacilli* spp.** | **Zone of inhibition (in mm)** | | | |
|---|---|---|---|---|
| | ***S. typhi*** | ***S. aureus*** | ***H. pylori*** | ***P. aeruginosa*** |
| ***L. casei*** | 11.70 | 14.50 | 17.74 | 15.20 |
| ***L. plantarum*** | 13.35 | 14.40 | 15.80 | 16.45 |
| ***L. acidophilus*** | 14.20 | 13.20 | 14.70 | 14.50 |
| ***L. bulgaricus*** | 14.60 | 14.50 | 13.90 | 14.00 |
| ***L. salivarious*** | 14.90 | 14.40 | 13.40 | 14.60 |
| ***L. delbrueki*** | 15.10 | 14.70 | 14.20 | 14.80 |
| ***L. brevis*** | 14.10 | 12.70 | 13.40 | 13.10 |

**Table 6.** Comparison of average inhibitory effects of *Lactobacilli* against pathogens for both methods

| ***Lactobacilli spp.*** | **Average inhibitory action of *Lactobacilli spp.* against all pathogens** | |
|---|---|---|
| | ***Well-diffusion*** | ***Disk-diffusion*** |
| ***L. casei*** | 13.74 | 14.79 |
| ***L. plantarum*** | **13.99** | 15.00 |
| ***L. acidophilus*** | 13.45 | 14.15 |
| ***L. bulgaricus*** | 13.46 | 14.25 |
| ***L. salivarious*** | 13.38 | **15.10** |
| ***L. delbrueki*** | 13.85 | 14.70 |
| ***L. brevis*** | 13.58 | 13.32 |

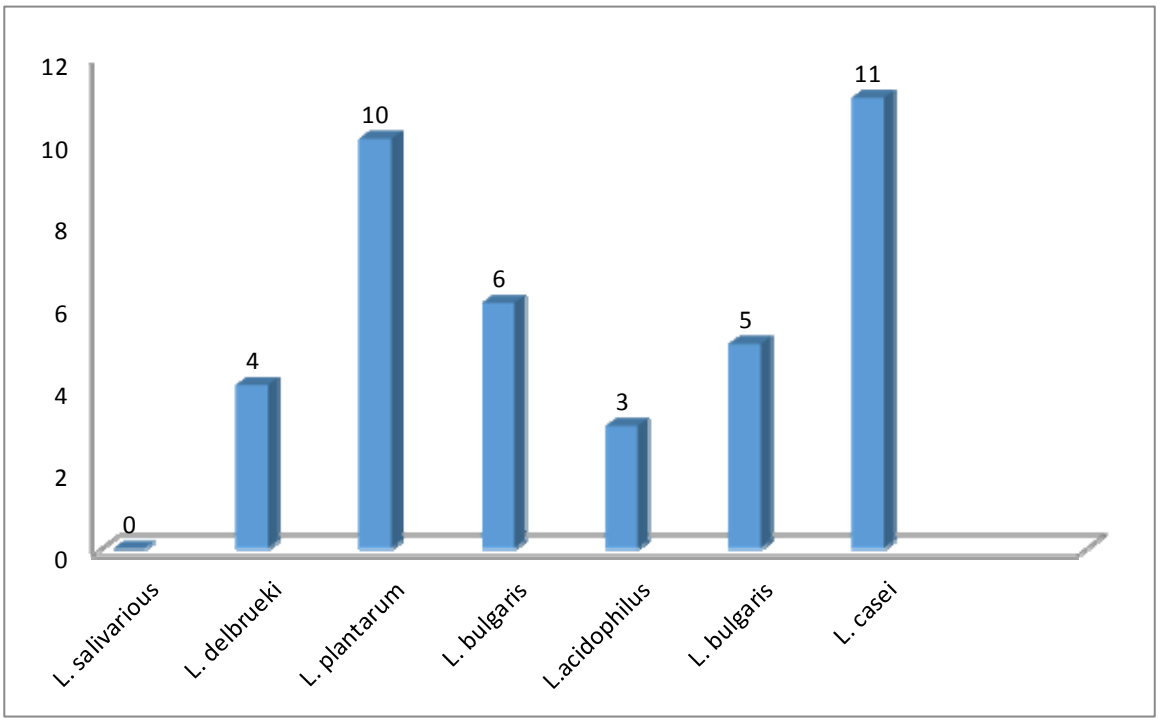


**Figure 1.** Frequency of *Lactobacillus spp*. isolated from different samples

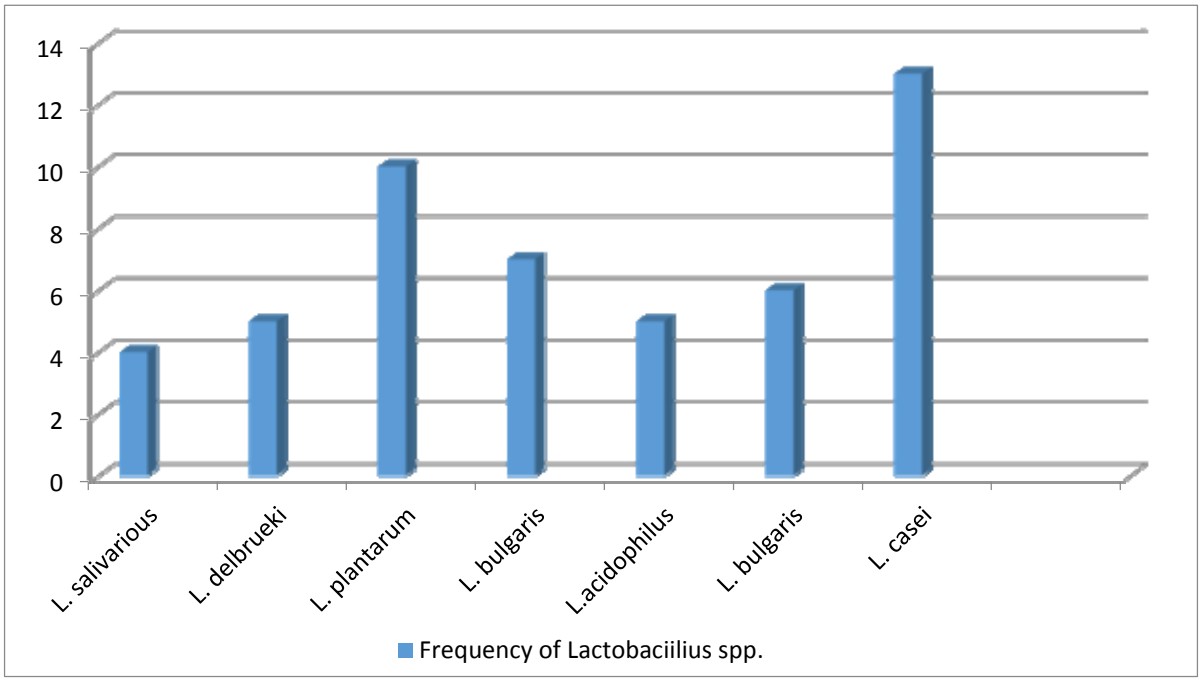


**Figure 2.** Frequency of inhibitory *Lactobacillus spp*. in different samples

Fastidious microorganism such as *Lb. helveticus*, *Lb. delbrueckii subsp* and *L. bulgaricus*, were grown through specific fermentation process. Lactic acid bacteria contained *Lb. plantarum* as a dominant member. Other isolates like *Ln. mesenteroides*, and *Lb. brevis* were also observed. Species isolated from goat milk of four Algerian races were *Lb. plantarum*, *Lb. brevis*, *Lb. helveticus*, *bulgaricus* and *delbrueckii subsp* (Tserovska et al., 2002).
In another study, LAB isolated from raw milk is used in bio preservation of cheese, yogurt, cream and other fermented foods. Sugar fermentation test and acid production were performed and showed the organoleptic properties. Bacteriocin producing strains like *mesenteroides* FR 52 and *Leuc. mesenteroides sp*. were identified which showed inhibiting activity towards *Enterococcus*, *Leuconostoc* strains and *Listeria spp*. All isolated strains of LAB are generally regarded as safe (GRAS) due to their linking with qualified Presumption of safety (Silva et al., 2013). *Enterococci* caused the abnormal colour, softening, splits and cracks for dairy products like cheese yogurt and butter. It is isolated from mentioned fermented dairy products. Other predominant lactic acid bacteria were *E. faecium*, *E. hirae*., *E. faecalis,* and *E. durans*. *Enterococci* was resistance to several antibiotics due to biofilm formation (Manzoor et al., 2016).

Another study was conducted on the isolation of *Lactobacillus spp*. from dairy products used in fermentation. Antibacterial activity of *Lactobacillus spp*. was performed against clinical pathogens such as *Salmonella typhimurium* (4.3 mm), *Enterotoxigenic E. coli* (4.2 mm) and *L. monocytogenes* (5.0 mm). Study isolates *S. typhimurium* and *E. coli* showed similar antagonistic activity than *Lactobacillus salivarius* and *Lactobacillus plantarum* isolated (De Keersmaecker et al., 2006).

The high antibiotic resistance exhibited by the uropathogenic *Staphylococcus spp*. were highly resistance to aminoglycosides due to modification of hydroxyl or amino group of aminoglycosides with the help of enzymes. 67% uropathogenic bacteria belong to *Enterobacteracea* family were resistance levofloxacin and ciprofloxacin, co-trimoxazole (TMP-SMX) resistance was also mentioned in gram-negative bacteria (Thomer et al., 2016). Microorganisms become resistance due to gene mutation and enhancing mobile genetic elements to develop resistance genes. Antibiotics class belong to Aminoglycoside (AG) such as gentamicin and amikacin used to treat Gram-positive and Gram-negative bacterial infections

respectively. Bacteria like; *S. aureus* and *E. coli* had shown multi-drug resistance. Amikacin resistance is developed in the bacteria due to cell wall thickening. The strains used in study, exhibited resistance to antibiotics such as ciprofloxacin, teicoplanin, linezolid, vancomycin, gentamicin, chloramphenicol and ceftizoxime. All isolates were MDR while MRSA was resistance to gentamicin and amikacin (Exner et al., 2017; Koulenti et al., 2019).

Foodborne pathogens cause gastroenteritis in human and animals. The prominent is *Salmonella enterica* in all of them. This bacterium caused severs gastroenteritis that leads to systemic infections such as typhoid fever. The death rate is increasing in human due to salmonellosis globally. While bacteria also caused non-typhoidal *Salmonella* in newborn babies having a weak immune system to cause invasive infections (Mathur et al., 2012). *Bovine salmonellosis* in animals reduced the economic rate due to productivity reduction, loss of weight, low milk and meat production. Many antibiotics have been used to overcome salmonellosis. But bacterium is becoming resistance due to long time usage and ultimately intestinal microflora disturbed caused gastrointestinal infections.

In this study, 68% antibiotics resistance were observed in enterococcal strains, 71% *E. faecium*, and 62% *E. faecalis*, *E. faecalis* was highly resistance to rifampicin and ciprofloxacin. 45% *E. faecium* was resistance to tetracycline and erythromycin but 5% in *E. faecalis* strain. *Enterococci* isolated from water areas showed sensitivity to penicillin, vancomycin, ampicillin and gentamicin (Manzoor et al., 2016). *Pediococcus Lactobacillus* and *Weissella* genera were 44, 33 and 60% respectively. Besides this, *lactococci* and *leuconostocs* genera did not show such characteristics. Mostly, 37% *Enterococcus* genus especially *E. faecalis* was resistance to multiple antibiotics than 5% non-enterococcal strains (Duncan et al., 2002). Lactic acid bacteria (LAB) isolated in the conducted study showed prominent antibiotics activity against *Klebsiella spp* and *E. coli* isolated from different sources such as, swine feces, canine feces, human faeces, boiler meat samples and wild waterfowl faeces. *E. coli* caused complicated urinary tract infections which were treated by *Weisella confusa* present in cow (Ayeni et al., 2009).

Thermophilic lactic acid bacteria including *L. helveticus* and *L. delbrueckii* showed prominent growth at 45°C and used in the production of different types of cheese like hard and Swiss cheese. Proteolytic activity was also reported in *L. helveticus* which produced different peptides that promote health. Other characteristics of lactic acid bacteria or probiotics were also observed (Duncan et al., 2002). *Bifidobacterium spp*. and *Lactobacillus spp*. were reported for coagulation of milk. These strains showed an antimicrobial activity against *E. coli*, *Vibrio cholerae*, *shigella* species and *Salmonella typhi*. Zone inhibition range was 10 mm to 22 mm diameter. That means isolated probiotic strains can produce antimicrobial product which can restrain the growth of pathogenic bacteria.

**Conflict of Interest:**

There is no conflict of interest.

**Refereces**

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