Canrea Journal: Food Technology, Nutritions, and Culinary, 2023; 6 (1): 42–56 OPEN ACCESS

e-ISSN : 2621-9468

Type of the Paper (Article)

Isolation and some basic characteristics of lactic acid bacteria from beetroot (*Beta vulgaris L.*) —A preliminary study

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Abstract

The carbohydrate content of fruit and vegetable juices has made them a suitable environment for the growth of lactic acid bacteria. Various studies have shown that beetroot can be a good substrate for the production of probiotic drinks for consumers. Therefore, this study was performed to isolate and identify lactic acid bacteria from beetroot juice. Beetroot juice was prepared from two kilograms of washed beetroot with a cold press. Sterile samples of beetroot juice were first enriched in MRS broth and then cultured on MRS agar. Gram and catalase tests were used for the initial detection of lactic acid bacteria. Then, to identify the species of lactic acid bacteria, biochemical confirmation tests such as the melting of gelatin, and the growth of bacteria has been studied in the ideal condition that is 15-45° C, sugars fermentation, gas production from glucose, and growth in at different concentrations of salt were performed. The final verification of species was done using polymerase chain reaction-amplified 16S rRNA-specific DNA. All isolates formed creamy white round colonies on MRS agar medium, were gram-positive, were able to grow in anaerobic conditions, and were also rod-shaped and catalase-negative. Based on biochemical tests, four types of lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus salivarius*, and *Lactobacillus acidophilus*) were identified. The findings of this study indicated the presence and diversity of lactic acid bacteria in beet juice, which can be a potential platform for the production of probiotic beetroot juice as a non-dairy product.

Article History
Received February 10, 2023
Accepted June 1, 2023
Published June 26, 2023

Keywords
Lactic Acid Bacteria,
Beetroot Juice,
Isolation,
Biochemical Identification,
Molecular Identification.

1. Introduction

Today, food must meet the nutritional needs of all population groups in terms of quantity, quality, and safety to attract consumers' attention (1). The World Health Organization (WHO) recommends that adequate amounts of antioxidant-rich vegetables and fruits should be included in daily diet to prevent diseases such as high blood pressure and coronary heart disease, and stroke, and also provide various health benefits (2,3). Since fruit and vegetable drinks have such beneficial properties and also contain fewer allergens and sensitizers than dairy products (4), researchers have focused on these drinks.

According to the WHO definition, a medicinal plant contains a substance that can be used for therapeutic purposes or which is a precursor for the synthesis of useful drugs (5,6). Beetroot (*Beta vulgaris* L.) is a rich source of vitamin C, magnesium, sodium, potassium, betaine, and vitamins B_6, and B_{12}, and it is the 10^{th} most powerful vegetable in terms of antioxidant properties (4). Beetroot is widely used due to its medicinal properties (7). Beetroot drinks can prevent infectious and malignant diseases (8). As red beetroot has a high nutritional value, is abundant in the country at a reasonable price, and also disposal of excess

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ISSN 2621-9468 online, DOI 10.20956/canrea.v6i1.980

fruits and vegetables due to not using proper harvesting methods and post-harvest technologies, processing beetroot extract as a beverage is very effective in increasing the nutritional value and reducing its waste (9). After production, both probiotics and starter cultures may be added to raw products to start or accelerate the fermentation process**.** Bacteria, yeasts, and molds can be used as a probiotic and starter culture**.** Naturally, these microorganisms exist or are added to many foods (10, 11). They have enzymatic activity, these microorganisms create desired properties (such as taste and texture) in the final product (12). Lactic acid bacteria (LAB) are considered starter cultures.

Lactic acid bacteria (LAB) include a wide range of genera such as *Lactobacillus, Pediococcus*, *Lactococcus*, *Loconostoc*, and *Streptococcus*. Of these genera, Lactobacillus is the largest group and includes more than 110 species(13). These bacteria share many biochemical, physiological, and genetic characteristics (14-16). LAB are gram-positive microaerophilic rod-shaped or cocci that have low G + C and do not produce spores (14,17). LAB also produces lactic acid as the main end product of carbohydrate fermentation and is predominant in fermented foods (14,17). LAB are catalase-negative and homo or heterofermentative bacteria that grow under low acid conditions (14). LAB are generally recognized as safe (GRAS) (14). LAB have been used as a starting culture in many foods because they increase the acceptability, nutritional value, and shelf life of fermented products and also improve their flavor and microbial quality (14,18). It is also worth noting that LAB can produce an antimicrobial compound known as "bacteriocins", which is used as a natural bio-protector against food-borne pathogens (17). They also produce a variety of antimicrobials such as lactic acid (and other organic acids), hydrogen peroxide, and antifungal peptides that prevent food spoilage and pathogenic bacteria (14). LAB has other beneficial effects, including preventive properties against intestinal infections (19), anti-cancer activity (20), serum cholesterol balance (21), and increased animal growth (22). Many studies show that consuming products containing probiotics and lactic acid bacteria regulates microflora, stimulates and develops the immune system, and improves intestinal health. LAB is found naturally in many foods, including dairy, cereals, meat, fish, fruits, juices, pickled vegetables, sourdough, water, and foodsheds, as well as in animals and the human digestive tract. LAB is also found naturally at various levels in different plants, including medicinal plants that contain LAB strains in large quantities, and on decaying plant materials, especially rotten fruits (5,23). Usually, the most powerful means to obtain useful and stable strains of microorganisms for use in industrial products has been the isolation and screening of these microorganisms genetically from natural sources (24). Previous studies have confirmed the isolation of a large number of different LAB strains from various plant sources. These findings have motivated us to choose an herbal drink prepared from beetroot juice. The results of this study can further confirm the results of previous studies and on the other hand, such positive results can increase the use of healthy natural food products and encourage consumers to incorporate such valuable foods into their eating habits (25).

Although beetroot juice is available as a beverage product, very limited information is available on its associated LAB. Even though biotechnological studies in the food industry have recently designed several beetroot juice products that contain a probiotic starter strain or LAB (13, 26-28), there are no studies on the LAB content of natural strains.

Therefore, in this study, we intended to differentiate and identify LAB strains isolated from natural beetroot juice using molecular and biochemical methods, to provide insight into the presence and diversity of LAB associated with beetroot juice. These isolates can be used

as starter cultures in the production of dairy and non-dairy functional foods. To achieve the purpose of this study, after preparing beet juice and culture on MRS culture medium, biochemical tests including the melting of the gelatin, the growth of bacteria has been studied in ideal conditions that are 15-45 degree Celsius, fermentation of sugars, gas production from glucose, and growth in different concentrations of salt, as well as PCR molecular testing was performed.

2. Materials and Methods

2.1. Isolation and Purification of Strains

LAB was isolated according to international standard methods (29,30). Sample collection was performed all around the Khorasan Razavi State. Beetroot samples were collected from different grocery/vegetable stores located in Mashhad City. The study was conducted from September 2022 to December 2022. Samples were collected in sterilized polybags and brought to the laboratory within 24 h and stored in a refrigerator at 4°C until the analysis. Then the samples were washed with drinking water, peeled, and cut into small pieces. 10 ml of beetroot juice was prepared from two kg of washed beetroot samples of similar size and free from any external defects, using cold pressure juicing, which is a recently developed fast extraction method to fulfill the growing demand of consumers. Freshly prepared cold-pressed juice contains all the natural nutrients because in this method, crushing and pressing of the product is performed without generating heat, and therefore, preserves the nutrients. Sterile samples of beetroot juice were enriched in 40ml of sterile MRS broth (Merk, Germany) for 24 hours at 37 °C under anaerobic conditions (Gas-Pack, BBL, Merk, Germany) (31).

A pour plate culture was then performed in sterile MRS agar (Merk, Germany) at 37 °C for 48 hours to isolate bacteria of the beetroot juice (31). LAB was phenotypically recognized based on morphological and biochemical characteristics; Gram staining, catalase activity, CO_2 production from glucose, and fermentation of sugars (sucrose, mannitol, rhamnose, sorbitol, and maltose), as well as growth at 15 and 45 °C and specific NaCl concentrations. The use of a phenol red broth culture medium, which is the basis for carbohydrate fermentation, is recommended (25). After incubation, several colonies were removed from each plate and examined by Gram staining, and catalase test (3). The typical morphological appearance of the colonies was small creamy white points, which were Gram-positive, catalase-negative, and rod-shaped (32).

The desired single colonies (Gram-positive and catalase-negative) were cultured linearly on new plates containing MRS agar medium and were incubated for 48 hours under anaerobic conditions at 37 °C.

2.2. Biochemical Analysis

2.2.1. Catalase Test

The catalase test is used to detect the ability of isolates to produce the enzyme catalase, which releases oxygen from hydrogen peroxide. Gas production indicates positive and no gas production indicates a negative test (33). A drop of 3% hydrogen peroxide solution was applied to each isolate to check for catalase activity. As soon as bubbles started to develop, catalase was present in the cells. All Gram-stained isolates were catalase-negative (24).

2.2.2. Gram Staining

When bacteria are stained with a primary dye (crystal violet), the bacteria will absorb the dye or will release the dye. After rinsing with alcohol and safranin, bacteria will absorb dyes. Subsequently observed under a microscope, the Gram-positive test indicated that the bacterial cells would be colored purple, while the Gram-negative bacterial cells would be pink (34).

2.2.3. Gelatin Hydrolysis

To perform the gelatin melting test, slant culture was performed in tubes containing nutrient gelatin (Merck, Germany) incubated at 37 °C for 24 hours following refrigeration at 4 °C for 1 hour. An uncultivated tube was also incubated and refrigerated as a control (35).

2.2.4. Growth at 15 and 45 °C

The ability of bacteria to grow in MRS broth at 15 and 45 °C was also examined (35). LAB cultures (1% v/v) were incubated for 24 hours overnight in MRS broth at 15 and 45 °C, respectively. The turbidity of their growths was then measured with a spectrophotometer at 600 nm, the growths were then seeded on MRS agar plates and incubated for 24-48 h at 37 °C. The appearance of LAB colonies matches with and supported the growth of those colonies in MRS broth (26).

2.2.5. Carbohydrate Fermentation Test

Phenol Red Broth Base (Merck, Germany) was prepared and 20µl of 5% sugar solution sterilized with a 0.22 µm filter was added to each phenol Red Broth Base medium containing inverted Durham. After inoculation at 37 °C under anaerobic conditions for 72 hours, a color change from red to yellow was reported as a positive result (36). The utilized carbohydrates were: glucose, sorbose, xylose, sucrose, lactose, trehalose, mannitol, and sorbitol (37).

2.2.6. Salt Tolerance

NaCl as an inhibitor may inhibit the growth of certain types of bacteria, and probiotics must be capable of resisting the concentration of salt in the human intestine (38). To evaluate the salt tolerance, the bacteria isolated from the fresh medium were inoculated in MRS broth media with different concentrations of sodium chloride salt (1, 3, 7, and 9%) and were incubated at 37 °C for 72 hours. The results were then examined by spectrophotometry and the absorbance at 600 nm was used to evaluate the viability of the strains (39). The results of the identified isolates were matched with the biochemical properties of lactic acid bacteria (LAB) in Bergey's book (23).

2.3. Molecular Identification by 16S rRNA Sequencing

2.3.1. DNA Extraction

Total nucleic acid units were extracted from MRS agar plates using Sinaclon commercial DNA extraction kit (SinaClon Co., Iran). The DNA extraction method was performed according to the manufacturer's instructions. The concentration of extracted DNA was measured using a Nanodrop spectrophotometer (Thermo Fisher Science Co., USA). The purity of the extracted DNA sample (260/280 ratio) was between 1.82 and 1.96.

2.3.2. Polymerase Chain Reaction and 16S rRNA Gene Amplification

To perform the polymerase chain reaction (PCR), general primers (Pishgam Biotechnology Co., Iran) with the following genome sequence were used:

Forward: 5'-AGC AGT AGG GAA TCT TCC A-3'
Reverse: 5'- CAC CGC TAC ACA TGG AG-3'

PCR was performed using a PCR thermocycler (Bio-Rad, America). The final volume of PCR solution for the final detection and confirmation of bacterial lactic acid was considered equal to 25 µl. So that 12/5 µl Mastermix (Applied Biosystem, America), 1 µl initial primer, 1 µl end primer, 5 µl distilled water, and 5.5 µl DNA were poured into each microtube. The temperature program is as follows in Table 1 (40):

Table 1. PCR temperature program.

Primary denaturation	Denaturation	Annealing	Extension	Final Extension	Number of cycles
95 5 minutes	95 1 minute	60 30 Seconds	72 1 minute	72 5 minutes	30

2.3.3. Separation of Amplified Fragments by Electrophoresis

Then the PCR product was placed on agarose gel (Merk, Germany), and electrophoresis (England, Cleaver Scientific Ltd., Cleaver) was used to separate the amplified PCR fragments. To prepare the gel (1%) and perform electrophoresis, 2 cc of 1x TAE buffer was added to 0.2 g of agarose (to prepare 10x buffer, 48.4 g of tris-base, 14.1 ml of glacial acetic acid and 0.37 g Na_2EDTA mixture, then the volume of the solution was made up to 100 cc with distilled water) after that the total volume was made up to 20 cc and placed on a heater until the agarose was completely dissolved and the solution became completely clear. After the solution was slightly cooled, 1.2 µL of green Viewer gel dye (Parstous, Iran) was added and the Arlene content was poured into the tray slowly so that no bubbles were formed. Then, the gel tray containing coagulated agarose was placed horizontally in the electrophoresis tank after removing the comb. 1x TAE buffer was slowly poured into the tank to cover at least a few milliliters on the surface of the gel. From all the PCR samples, 4 µL were removed and mixed with 2 µL of 100bp DNA Ladder Ready (Yekta tajhiz azma Co., Iran) in the loaded gel wells. The electrophoresis tank was connected to the power source so that the wells were toward the negative pole. The voltage of the electrophoresis device was set to 60 for at least 35 minutes. Then, the gels were placed in the gel dock machine and the band formation at 300-400 bp was examined (41).

3. Results and Discussion

3.1. Isolation and Purification

A total of 4 LAB were selected out of total colonies that grew on MRS agar plates after incubation for 48 hours at 37 °C, based on specific morphology (small point and creamy white colonies), and were characterized as Gram-positive, catalase-negative, and rod-shaped (Figure 1-2).

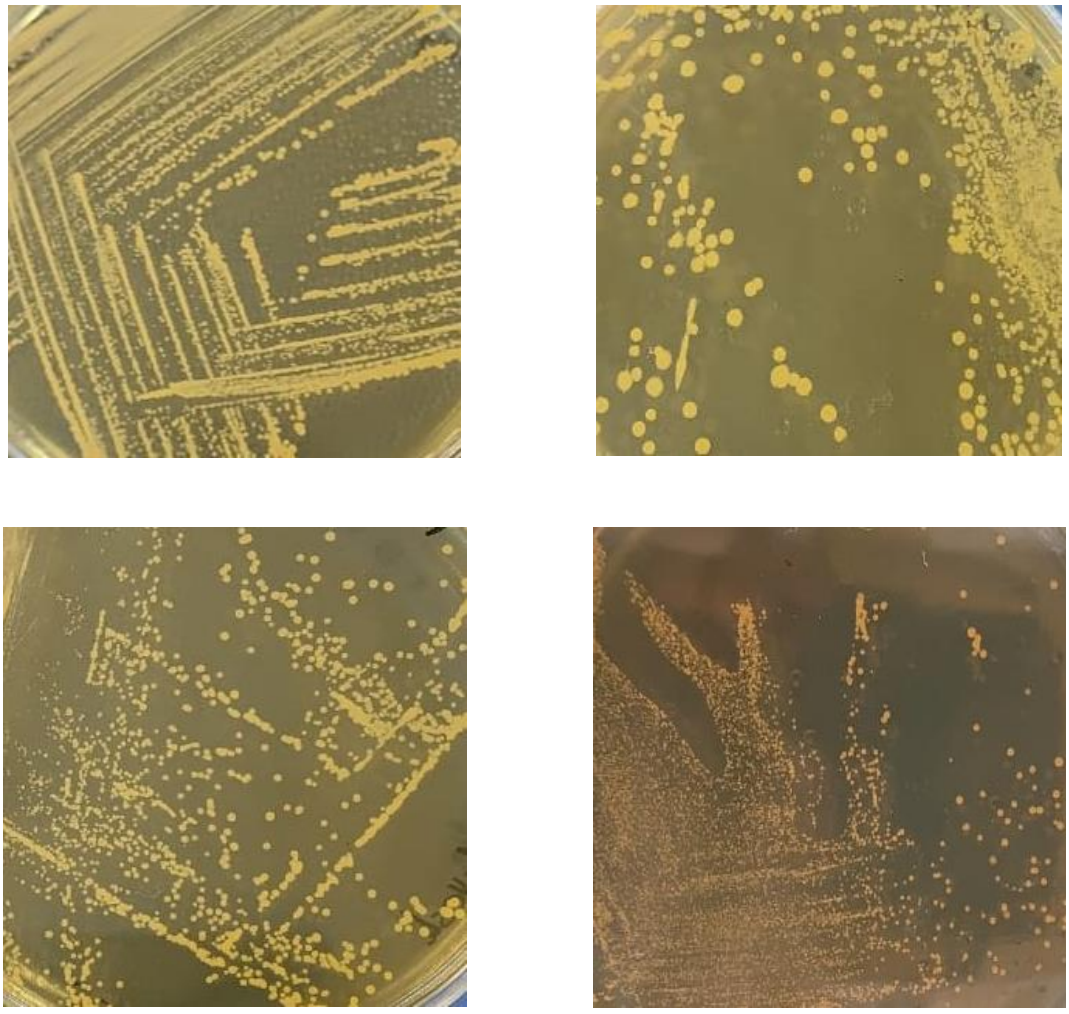
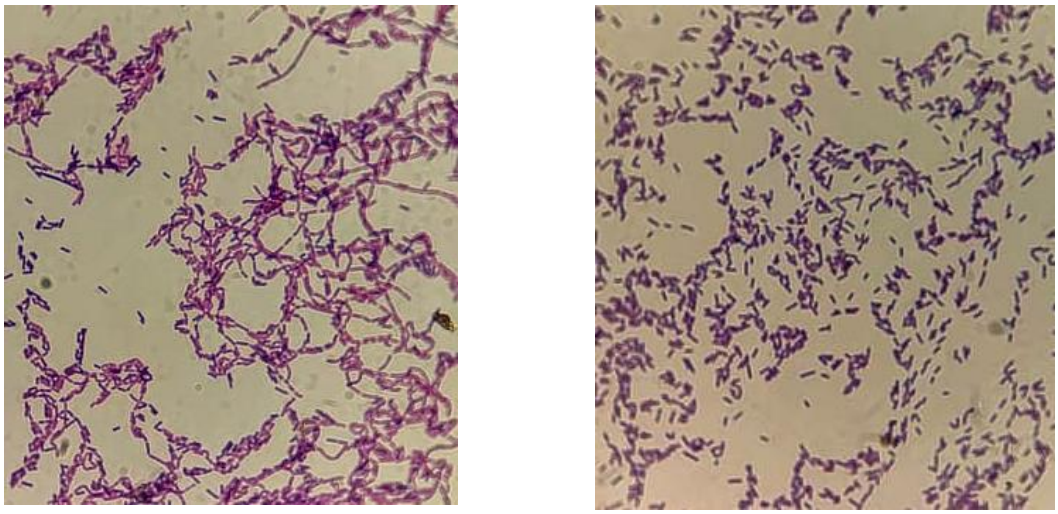


Figure 1. Colony morphology, the desired colonies were cultured on MRS agar plates and incubated for 48 hours under anaerobic conditions at 37 °C.



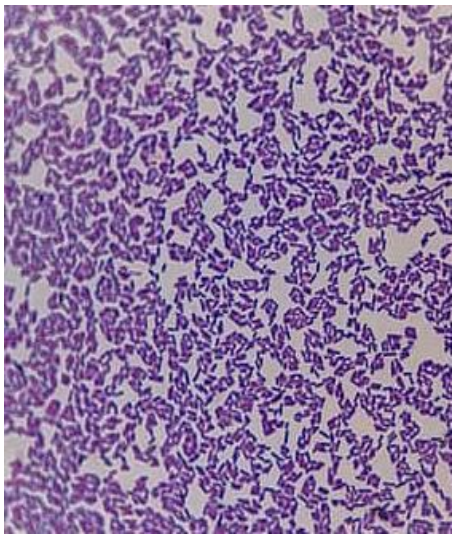
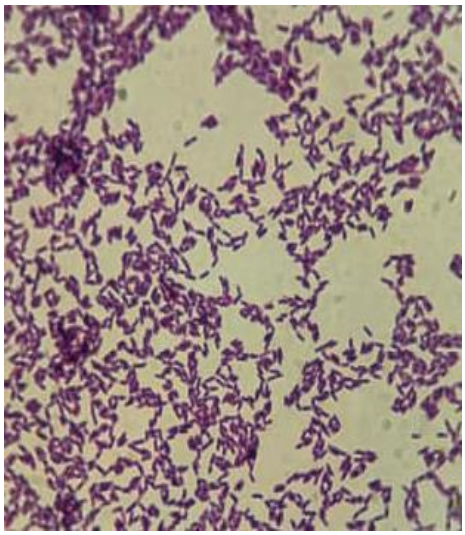



Figure 2. Optical microscope image (× 40) of isolates, 4 lactic acid bacteria (LAB) were selected based on Gram-positive staining, and rod-shaped.

3.2. Biochemical Identification of Isolated Bacteria

To eliminate potentially harmful bacterial strains, gelatin analysis is one of the tests performed to determine the safety status before selecting bacterial strains for use as probiotics in humans, so isolates that do not show gelatin degradation are considered safe (42).

None of the isolated bacteria were able to melt the gelatin. This was consistent with the 2019 paper, which found that none of the LAB strains exhibited gelatin hydrolysis activity. The hydrolysis of the gelatin indicates the proteolytic activity of the LAB, therefore the detection of the hydrolysis of the gelatin is important for the selection of probiotic strains of the LAB (35).

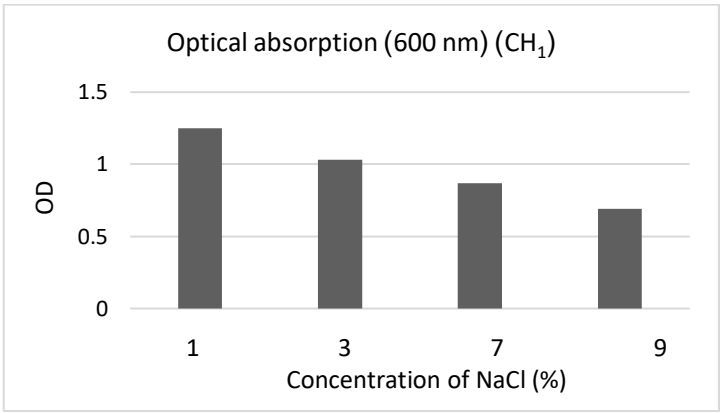
Results of the 8 sugars fermentation test and growth at 15 and 45 °C that were used for further identification are shown in Table 2.

Table 2. Carbohydrate fermentation test of isolated strains.

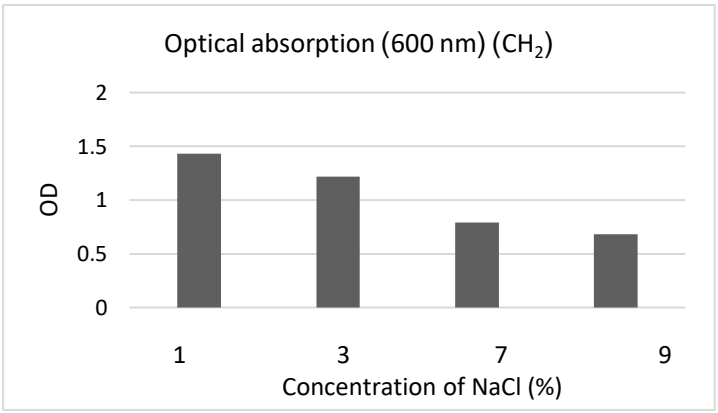
Bacteria \ Sugar	Glucose	Lactose	Sucrose	Xylose	Sorbose	Trehalose	Sorbitol	Mannitol	Catalase	Gas production from glucose	Grow at 45 °C	Grow at 15°C
CH_1	+	+	+	+	-	+	+	+	-	-	+	-
CH_2	+	-	-	-	+	+	-	-	-	-	+	-
CH_3	+	+	+	-	+	+	+	-	-	-	+	-
CH_4	+	+	+	-	-	+	+	-	-	-	+	-

Results showed that all of the isolates could not produce carbon dioxide from glucose. One of the characteristics of heterofermentative lactobacilli is the production of gas from glucose, which ferments hexoses into lactic acid, acetic acid, or ethanol and carbon dioxide (35). As stated in the studies, homofermentative strains such as *L. plantarum*, *L. helveticus*, and *L. acidophilus* cannot produce carbon dioxide from glucose. Furthermore, strains such as *L. acidophilus* and *L. helveticus* can grow at 45 °C but not at 15 °C (43).

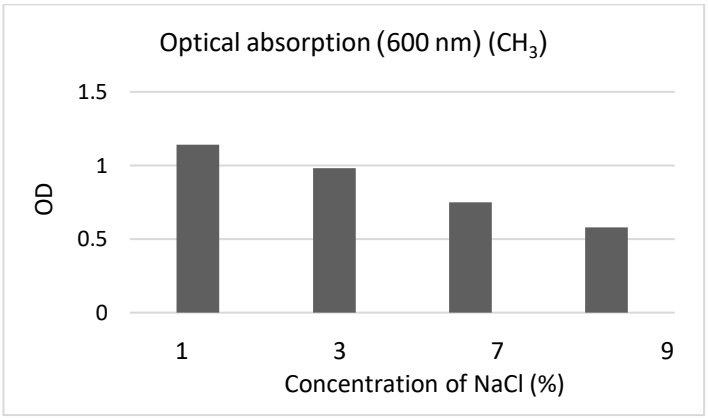
The optical absorption of the isolates was obtained after 72 hours of incubation at 37 °C in salt stress environments at 600 nm (Figure 3).



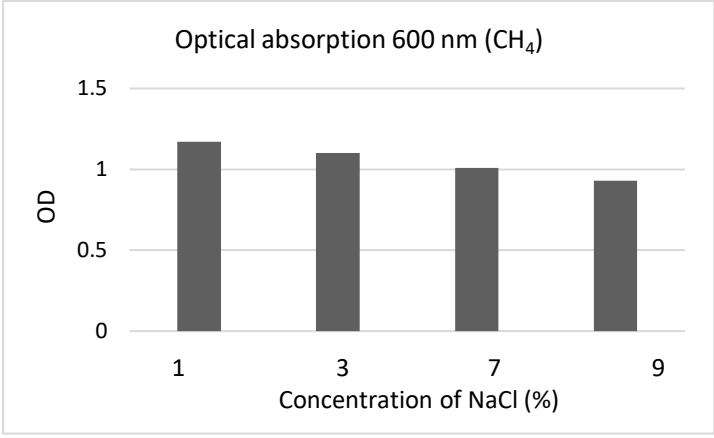
(a)



(b)



(c)



(d)

Figure 3. Chart of maximum growth of isolates (a) CH_1; (b) CH_2; (c) CH_3; (d) (CH) in different NaCl concentrations after 72 hours.

Isolates were able to resist and grow at salt concentrations of 1% and 3% and grew well in these media. Bacterial growth rate and resistance decreased in salt concentrations of 7% and 9% in all isolates and did not decrease significantly in the CH_4 isolate. All the isolates were present at counts higher than 10^5 CFU/g. In a study conducted in 2019, most strains such as *L. plantarum* were able to grow at 6.5% NaCl, but not at 10% NaCl (28). Another study in 2019 also showed that the isolates of Lactobacillus had resistance to different concentrations of NaCl (1–6%) and were capable of being probiotics. *Lactobacillus spp.* isolated in this study were able to tolerate 1–9% of NaCl, with good growth observed at 1–5% NaCl (38).

3.3. Molecular Identification of Isolated Bacteria

Identification (at the genus level) of isolates is one of the most important applications of PCR. After amplification of 16s rRNA gene using PCR reaction for DNA extracted from desired isolates, the amplicons obtained were purified and the final product was obtained on the agarose gel.

Figure 4 shows the electrophoresis result of the final product purified from the 16s rRNA gene amplification using PCR. The length of the obtained bands was within 320-400 bp, which confirmed the result of the identification of bacteria at the genus level (Figure 4).

The determination of selected isolates species was done using biochemical tests, which revealed four species, including CH_1=*Lactobacillus plantarum*, CH_2=*Lactobacillus helveticus*, CH_3=*Lactobacillus salivarius*, and CH_4=*Lactobacillus acidophilus*.

Apart from our study, another study in 2022 that isolated LAB from jackfruit and beetroot juice used molecular tests reported that the LAB isolated were *Lactiplantibacillus plantarum* and *Levilactobacillus brevis*, respectively (44).

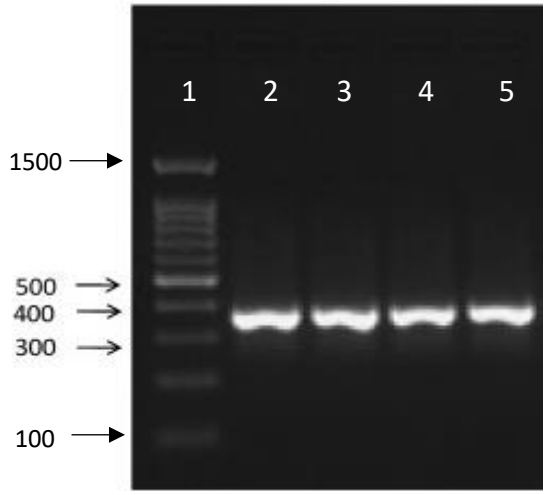


Figure 4. PCR product of 16s rRNA (320-400 bp) from 4 isolates that are reproduced using universal primers. Row 1: DNA marker (100 DNA Ladder), row 2: *L. plantarum*, row 3: *L. helveticus*, row 4: *L. salivarius*, row 5: *L. acidophilus*.

Finally, the colony color of LAB isolates varied from pale white to cream. The cell shape of LAB was a rod seen under a microscope (40X). All LAB isolates were gram-positive and catalase-negative. The results of sugar fermentation and growth at temperatures of 15 and 45 are presented in Table 2. The microbial population was also estimated to be 10^5.

The popularity of probiotic cells in food products has attracted consumers and the industry has taken into account the requirement to identify good sources and survivability of lactic acid bacteria (as good probiotic strains). From this point of view, beetroot is a good source of nutrients and antioxidants and its natural sugar content makes it a probiotic powerhouse (26).

The high content of *L. plantarum* after 21 hours in beetroot juice was reported to be attributed to the biological compounds and plant matrix nutrients that somehow protected the bacterial cells and thus maintained their viability, which was in line with the findings of our study regarding the suitability of beetroot juice for the growth of lactic acid bacteria (26).

Various studies have shown the presence of lactic acid and probiotic bacteria, for example:

Maślak E et al. isolated LAB strains from pickled beetroot samples, which can be used in the dairy industry and were considered as a probiotic in the future (44). The two strains identified using the MALDI method were *Lactococcus lactis* and *Weissella cibaria* (45).

In a study in 2004, Inoculums for the fermentation of sugar beet juice were developed and three cultures of lactic acid bacteria were used: *L. acidophilus*, *L. plantarum*, and *L. delbrueckii*. Fermentation of the beet juice was performed for 5 to 7 days at 37°C for *L. acidophilus* and *L. delbrueckii*, and at 30°C for *L. plantarum*. Maximum accumulation of lactic acid in the beetroot juice was obtained after six days of cultivation. The studied lactic acid bacteria synthesized 67 to 69% of lactic acid (46).

In a study conducted in 2005, Red beets were evaluated as a potential substrate for the production of probiotic beetroot juice by four species of lactic acid bacteria (*L. acidophilus, L. casei, L. delbrueckii*, *L. plantarum*). All the lactic cultures were found capable of rapidly utilizing beetroot juice for cell synthesis and lactic acid production. However, *L. acidophilus and L. plantarum* produced a greater amount of lactic acid than other cultures and reduced

the pH of fermented beetroot juice from an initial value of 6.3 to below 4.5 after 48 h of fermentation at 30°C (27). In another study, the presence of lactic acid bacteria (*L. paraplantarum, L. pentosus*) in naturally fermented beetroot juice was confirmed using different molecular tools (47).

In a study in 2016, *L. casei* was used to produce a probiotic beverage containing apple, carrot, and beetroot juice. In this manner, fruit juice was mixed with lactic acid at a concentration of 10^6-10^7 CFU/ml, and fermentation was carried out for 48 hours at 37°C, which was considered to be a suitable temperature for the development of probiotic bacteria. The findings showed that the use of juice by probiotic bacteria decreased the amount of sugar present in the juice (48).

A study in 2017 was planned to prepare a non-dairy probiotic drink using beetroot juice. The probiotic potential was examined based on the viability of *L. rhamnnosus, L. plantarum,* and *L. delbrueckii* sb. The probiotic drink was prepared at pH 6.5 and an optimum fermentation temperature of 37 °C. The study showed that pH and sugar content gradually declined over time. The study revealed that the use of beetroot drinks is a good approach for non-dairy probiotics, as it is free from cholesterol and contains health-promoting components (4).

Results of many other studies evaluating beetroot stated that beets can be used as a suitable substrate to produce a healthy lactic acid drink for consumers who are lactose intolerant and vegans (13). In a study in 2019, the juice of red beets was extracted and fermented by two species of probiotic bacteria (*L. plantarum* and *L. paracasei*). Both species of lactic cultures could use beetroot juice for the production of lactic acid and the synthesis of cells. The fermentation process was carried out for 24 hours at 30°C by inoculating 24-hour cultures of *L. plantarum* and *L. paracasei* (10^6 CFU / ml) into 100 ml of beet juice. Following this period, a significant increase in cell count indicated that beetroot juice could solely provide a suitable substrate for the growth of lactic cultures (49). In our study as well, out of the typical colonies obtained from culture media, 4 colonies were identified as lactic acid bacteria (*L. plantarum, L. helveticus, L. salivarius,* and *L. acidophilus*).

Based on the results of previous studies and our study, it can be concluded that beetroot juice can act both as a substrate and a reservoir for lactic acid bacteria. Some studies have proven that four strains, including *L. plantarum, L. helveticus, L. salivarius*, and *L. acidophilus* are probiotics and have many benefits for human health. It is hoped that the probiotic properties of these isolates from beetroot juice will be proven in the future (50-53).

4. Conclusions

The isolated bacteria were identified as *Lactobacillus spp.* with the help of various morphological, and biochemical testing techniques, as per Bergey's manual. Morphologic identification was performed using the Gram staining test. Biochemical tests included catalase, carbohydrate fermentation, NaCl tolerance testing, and gelatine hydrolysis. We also used polymerase chain reaction for molecular confirmation.

The four LAB were identified by 16S rRNA gene sequencing from beetroot juice, which included four species *L. plantarum, L. helveticus, L. salivarius*, and *L. acidophilus*. Species confirmation was done using biochemical tests. The results of this study confirmed that beetroot juice was a good source of lactic acid bacteria, which according to the results of previous studies, is considered a suitable environment for the growth of these bacteria and provides useful information for scientific and commercial applications. Furthermore, beet-

based functional food products can be recommended for daily consumption, especially for lactose-intolerant people and vegetarians who cannot benefit from dairy probiotic products, which provide a high content of bioactive molecules as well as probiotic-related properties.

Acknowledgment

This article is taken from the results of the research project "Isolation and identification of lactic acid bacteria in beetroot juice" with code 4002206 at Mashhad University of Medical Sciences. We would like to express our gratitude to the Vice-Chancellor for Research of Mashhad University of Medical Sciences, who is the financial sponsor of this research project.

Author Contributions

This work was carried out with the collaboration of all authors who have reviewed and certified the final manuscript for submission.

Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of Interest

There are no conflicts of interest among the authors.

References

1. Nordhagen S, Lambertini E, DeWaal CS, McClafferty B, Neufeld LM. Integrating nutrition and food safety in food systems policy and programming. Global Food Security. 2022;32:100593.
2. Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, Norat T, et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality—a systematic review and dose-response meta-analysis of prospective studies. International journal of epidemiology. 2017;46(3):1029-56.
3. Pulipati S, Babu PS, Teja PS, Geethanjali P, Sri PV, Varma TR. Fermented veggies: antibacterial efficacy of isolated lactic acid bacteria. 2019.
4. Panghal A, Virkar K, Kumar V, Dhull SB, Gat Y, Chhikara N. Development of probiotic beetroot drink. Current research in nutrition and food science journal. 2017;5(3).
5. Saguibo J, Mercado M, Maldia S, Jimeno B, Perez M, Calapardo M, et al. Identification and characterization of lactic acid bacteria isolated from some medicinal and/or edible Philippine plants. Food Research. 2019;3(6):698-712.
6. Kuete V. Medicinal spices and vegetables from Africa: therapeutic potential against metabolic, inflammatory, infectious and systemic diseases: Academic Press; 2017.

7. dos S. Baião D, da Silva DV, Paschoalin VM. Beetroot, a remarkable vegetable: Its nitrate and phytochemical contents can be adjusted in novel formulations to benefit health and support cardiovascular disease therapies. Antioxidants. 2020;9(10):960.
8. Domínguez R, Munekata PE, Pateiro M, Maggiolino A, Bohrer B, Lorenzo JM. Red beetroot. A potential source of natural additives for the meat industry. Applied Sciences. 2020;10(23):8340.
9. Chen L, Zhu Y, Hu Z, Wu S, Jin C. Beetroot as a functional food with huge health benefits: Antioxidant, antitumor, physical function, and chronic metabolomics activity. Food Science & Nutrition. 2021;9(11):6406-20.
10. Santacroce L, Charitos IA, Bottalico L. A successful history: probiotics and their potential as antimicrobials. Expert Review of Anti-infective Therapy. 2019;17(8):635-45.
11. Vijaya Kumar B, Vijayendra SVN, Reddy OVS. Trends in dairy and non-dairy probiotic products-a review. Journal of food science and technology. 2015;52:6112-24.
12. Keba Wirtu A. Assessment of Dairy Food Environment, Physicochemical Properties, and Microbial Safety of Milk and Cottage Cheese across the Value Chain in Oromia Region, Ethiopia: Addis Ababa University; 2020.
13. Malik M, Bora J, Sharma V. Growth studies of potentially probiotic lactic acid bacteria (Lactobacillus plantarum, Lactobacillus acidophilus, and Lactobacillus casei) in carrot and beetroot juice substrates. Journal of Food Processing and Preservation. 2019;43(11):e14214.
14. Anacarso I, Bassoli L, Sabia C, Iseppi R, Condò C. Isolation and identification of lactic acid bacteria from plants and other vegetable matrices and microbial recombination with Enterococcus spp. Am Res Thoughts. 2015;1:1503-15.
15. Amelia R, Philip K, Pratama YE, Purwati E. Characterization and probiotic potential of lactic acid bacteria isolated from dadiah sampled in West Sumatra. Food Science and Technology. 2020;41:746-52.
16. Bintsis T. Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. AIMS microbiology. 2018;4(4):665.
17. Sowmya N, Nandini K, Earanna N, Sajeevan R, Nataraja KN. Molecular identification and genetic diversity of Lactobacillus species isolated from different edible sources. Journal of Pure and Applied Microbiology. 2016;10(4):3155-62.
18. Zapasnik A, Sokołowska B, Bryła M. Role of Lactic Acid Bacteria in Food Preservation and Safety. Foods 2022, 11, 1283
19. Campana R, van Hemert S, Baffone W. Strain-specific probiotic properties of lactic acid bacteria and their interference with human intestinal pathogens invasion. Gut pathogens. 2017;9(1):1-12.
20. Garbacz K, editor Anticancer activity of lactic acid bacteria. Seminars in Cancer Biology; 2022: Elsevier.
21. Jurášková D, Ribeiro SC, Silva CC. Exopolysaccharides produced by lactic acid bacteria: From biosynthesis to health-promoting properties. Foods. 2022;11(2):156.
22. Werning ML, Hernández-Alcántara AM, Ruiz MJ, Soto LP, Dueñas MT, López P, et al. Biological Functions of Exopolysaccharides from Lactic Acid Bacteria and Their Potential Benefits for Humans and Farmed Animals. Foods. 2022;11(9):1284.
23. Tarique M, Abdalla A, Masad R, Al-Sbiei A, Kizhakkayil J, Osaili T, et al. Potential probiotics and postbiotic characteristics including immunomodulatory effects of lactic acid bacteria isolated from traditional yogurt-like products. LWT. 2022;159:113207.

24. Sobrun Y, Bhaw-Luximon A, Jhurry D, Puchooa D. Isolation of lactic acid bacteria from sugar cane juice and production of lactic acid from selected improved strains. 2012.
25. Dosuky AS, Elsayed TR, Yousef ET, Barakat OS, Nasr NF. Isolation, identification, and application of lactic acid-producing bacteria using salted cheese whey substrate and immobilized cells technology. Journal of Genetic Engineering and Biotechnology. 2022;20(1):1-16.
26. Barbu V, Cotârleț M, Bolea CA, Cantaragiu A, Andronoiu DG, Bahrim GE, et al. Three types of beetroot products enriched with lactic acid bacteria. Foods. 2020;9(6):786.
27. Yoon KY, Woodams EE, Hang YD. Fermentation of beet juice by beneficial lactic acid bacteria. LWT-Food Science and Technology. 2005;38(1):73-5.
28. Rakin M, Baras J, Vukašinović-Sekulić M. The influence of brewer's yeast autolysate and lactic acid bacteria on the production of a functional food additive based on beetroot juice fermentation. Food Technology and Biotechnology. 2004;42(2):109-13.
29. Tabasco R, Paarup T, Janer C, Peláez C, Requena T. Selective enumeration and identification of mixed cultures of Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, L. acidophilus, L. paracasei subsp. paracasei and Bifidobacterium lactis in fermented milk. International Dairy Journal. 2007;17(9):1107-14.
30. Organisation IS. Milk Products–Enumeration of Presumptive Lactobacillus acidophilus on a Selective Medium–Colony-Count Technique at 37 Degrees C. ISO 20128: 2006 (IDF 192: 2006). International Standards Organisation Geneva, Switzerland; 2006.
31. Reuben RC, Roy PC, Sarkar SL, Alam R-U, Jahid IK. Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC microbiology. 2019;19(1):1-20.
32. Reuben RC, Roy PC, Sarkar SL, Alam R-U, Jahid IK. Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC microbiology. 2019;19:1-20.
33. Reiner K. Catalase test protocol. American society for microbiology. 2010:1-6.
34. Rahmawati N, Syukri M, Darmawi D, Zachreini I, Yusuf M, Idroes R, editors. Identification of lactic acid bacteria from etawa goat milk kopelma Darussalam Village, Banda Aceh. IOP Conference Series: Earth and Environmental Science; 2021: IOP Publishing.
35. Das AJ, Das MJ, Miyaji T, Deka SC. Growth and metabolic characterization of four lactic acid bacteria species isolated from rice beer prepared in Assam, India. Access Microbiology. 2019;1(4).
36. Nair PS, Surendran PK. Biochemical characterization of lactic acid bacteria isolated from fish and prawn. 2005.
37. Juliyarsi I, Hartini P, Yuherman DA, Arief PH, Aritonang S, Hellyward J, et al. Characterization of lactic acid bacteria and determination of antimicrobial activity in tempoyak from Padang Pariaman District, West Sumatra, Indonesia. Pakistan Journal of Nutrition. 2018;17(10):506-11.
38. Prabhurajeshwar C, Chandrakanth K. Evaluation of antimicrobial properties and their substances against pathogenic bacteria in-vitro by probiotic Lactobacilli strains isolated from commercial yoghurt. Clinical Nutrition Experimental. 2019;23:97-115.
39. Holt JG, Krieg NR, Sneath PH. Bergey's manual of determinative bacterology. 1994.
40. Taş TK, Ekinci FY, Guzel ‐ Seydim ZB. Identification of microbial flora in kefir grains produced in Turkey using PCR. International Journal of Dairy Technology. 2012;65(1):126-31.

41. Flusberg BA, Webster DR, Lee JH, Travers KJ, Olivares EC, Clark TA, et al. Direct detection of DNA methylation during single-molecule, real-time sequencing. Nature methods. 2010;7(6):461-5.
42. Khan AN, Yasmin H, Ghazanfar S, Hassan MN, Keyani R, Khan I, et al. Antagonistic, anti-oxidant, anti-inflammatory and anti-diabetic probiotic potential of lactobacillus agilis isolated from the rhizosphere of the medicinal plants. Saudi Journal of Biological Sciences. 2021;28(11):6069-76.
43. Harrigan WF. Laboratory methods in food microbiology: Gulf professional publishing; 1998.
44. Ashiwini G, Narayanaswamy B, Suvarna V, Munishamanna K, Suresh K. Characterization of lactic acid bacteria isolated from jackfruit and beet root juices for their probiotic properties. 2022.
45. Maślak E, Złoch M, Arendowski A, Sugajski M, Janczura I, Rudnicka J, et al. Isolation and identification of Lactococcus lactis and Weissella cibaria strains from fermented beetroot and an investigation of their properties as potential starter cultures and probiotics. Foods. 2022;11(15):2257.
46. Klewicka E, Motyl I, Libudzisz Z. Fermentation of beet juice by bacteria of genus Lactobacillus sp. European Food Research and Technology. 2004;218(2):178-83.
47. Kingston J, Radhika M, Roshini P, Raksha M, Murali H, Batra H. Molecular characterization of lactic acid bacteria recovered from natural fermentation of beet root and carrot Kanji. Indian Journal of Microbiology. 2010;50(3):292-8.
48. Zandi MM, Hashemiravan M, Berenjy S. Production of probiotic fermented mixture of carrot, beet and apple juices. 2016.
49. Jafar NB, Ghaleb ZT, Fadhil ZH. Production of fermented red beet juice using probiotic lactobacilli bacteria. Ann Trop Med Public Health. 2019;22:73-5.
50. Ramos CL, Thorsen L, Schwan RF, Jespersen L. Strain-specific probiotics properties of Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus brevis isolates from Brazilian food products. Food microbiology. 2013;36(1):22-9.
51. Foster L, Tompkins T, Dahl W. A comprehensive post-market review of studies on a probiotic product containing Lactobacillus helveticus R0052 and Lactobacillus rhamnosus R0011. Beneficial microbes. 2011;2(4):319-34.
52. Neville B, O'Toole P. Probiotic properties of Lactobacillus salivarius and closely related Lactobacillus species. Future Microbiology. 2010;5(5):759-74.
53. Tahmourespour A, Kermanshahi RK. The effect of a probiotic strain (Lactobacillus acidophilus) on the plaque formation of oral Streptococci. Bosnian journal of basic medical sciences. 2011;11(1):37.