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Evaluation of probiotic properties of *Lactobacillus brevis* as the predominant LAB isolated from fermented amaranth

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ABSTRACT

Evaluation of probiotic properties of lactic acid bacteria (LAB) isolated from fermented pseudocereals has crucial importance to prepare microbial cultures. In the present study, after molecular identification, probiotic properties of the predominant LAB isolate were investigated. Sequencing results of the PCR products led to the identification of *Lactobacillus brevis* SKA01 as the predominant LAB. The survival of the LAB isolate after continues treatment of acid and bile reached to 10^6 compared to the control sample (10^8 CFU/mL), and it showed the highest antibacterial effect on *Staphylococcus aureus*. Meanwhile, there was no significant difference (P>0.05) between inhibitory zone diameter of the *S. aureus* and *Listeria monocytogenes* in the present of the LAB isolate. LAB isolate was capable of good auto-aggregation (36.19%) and co-aggregation with *S. aureus* (71.24%), and it had no hemolytic activity. Furthermore, it was resistant to most of the tested antibiotics. By considering the proper probiotic potentials of the *L. brevis* isolated from fermented amaranth, it is possible to use the isolate as microbial starter or probiotic culture in fermentation industries.

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1. Introduction

Probiotics are live microorganisms that, when consumed in sufficient amounts, bring health benefits to the consumer [1]. Sourdough is a mixture of water and flour that is fermented randomly or by using a starter culture including lactic acid bacteria and yeasts and has interesting technological-functional capabilities. Lactic acid bacteria include a heterogeneous group of gram-positive, non-sporing, non-motile, aerobic, filamentous or cocci-shaped organisms that produce lactic acid as the main and final product during carbohydrate fermentation. There are also lactic acid bacteria with different origins and potential biological activities, among which Lactobacillus species are the most common [2].

amaranth (*Amaranthus*) belongs to the family*Amaranthaceae*It is a global genus of short-lived perennial plants that has approximately 60 species and originates from Mexico. This pseudo-cereal has lipids, proteins, carbohydrates and dietary fibers at a very high level. Also, there are important compounds such as tocopherols and phenolic compounds in amaranth, and the main fatty acids in amaranth oil include palmitic, oleic and linoleic. Also, amaranth has a high level of essential amino acids such as lysine and methionine, vitamins and minerals, especially calcium and magnesium [3].

So far, there have been studies on the microbial flora of fermented amaranth. For example, Sterr et al. (2009) isolated dominant lactic acid bacteria from random amaranth sourdough fermentation. Therefore, isolates*Lactobacillus plantarum*And*Pediococcus pentazaceus* With appropriate competitive ability, while rapidly reducing pH, it provides the possibility of constant and uniform fermentation at different temperatures, therefore, amaranth was chosen as a starter culture for sourdough [4]. In a research, Vera-Pingitore et al. (2016) investigated lactic acid bacteria isolated from quinoa and amaranth in terms of sensitivity to antibiotics, tolerance to unfavorable conditions of the digestive system and adhesion to intestinal epithelial cells, and out of 5 species that have a suitable pattern of resistance were antibiotics, used as probiotics [5]. Russo et al (2017) Antimicrobial activity of 88 subspecies*Lactobacillus plantarum* examined the screened samples and concluded that the high concentration of lactic acid produced during the growth phase of lactic acid bacteria with a decrease in pH significantly affects their antimicrobial properties [6].

Until now, the probiotic characteristics of the dominant lactic acid bacteria isolated from fermented pseudocereals have been less studied. Therefore, the aim of this study was to evaluate the probiotic characteristics of the dominant lactic acid bacteria of amaranth sourdough.

2- Materials and methods

2-1- Raw materials

To prepare amaranth flour, a specific variety of amaranth (*Bloody Amaranth*) was purchased from Kian Food Company (Iran) and it was prepared by an industrial flour mill and sieve with 50 mesh. The characteristics of the mentioned flour were determined based on documented methods (AACC, 2010) [7]. Microbial strains used include*Escherichia coli* (*Escherichia coli* PTCC 1399) ،*Staphylococcus aureus* (*Staphylococcus aureus* PTCC 1112) ، *Listeria monocytogenes* (*Listeria monocytogenes* PTCC 1298) and*Bacillus cereus*(*Bacillus cereus* PTCC 1015) was also purchased from the Iranian Scientific and Industrial Research Organization and then activated in suitable cultivation environments. Microbial culture mediums were obtained from Merck, Germany.

2-2- random fermentation of amaranth flour

At this stage, sourdough was prepared from amaranth flour with a dough yield equal to 200 (a mixture of 100 grams of amaranth flour with 100 milliliters of water). Then the mentioned dough was kept in a greenhouse at a temperature of 37 degrees Celsius for 24 hours. To isolate the dominant lactic acid bacteria, the fermentation process (adding 10% of the previous day's sourdough to the fresh dough) was continued until the pH of the sourdough reached about 4 [8].

2-3- Isolation of dominant lactic acid bacteria from amaranth sourdough

In order to achieve a pure monoculture of dominant lactic isolates, after preparing a

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surface culture from successive dilutions of sourdough in De Man, Rogosa and Sharpe agar (MRS) culture medium and studying the microscopic morphology of lactic isolates, finally a linear culture was prepared from them and warm tests and Catalase was performed on the isolates.

2-4- Molecular identification of dominant lactic acid bacteria

The DNA of dominant lactic acid bacteria was extracted using a commercial kit (Bionir, AccuPrep K-3032, South Korea) and amplified by PCR with F44 and R1543 primers, and then the PCR products were sequenced (Bionir Company, South Korea). The PCR reaction was performed in a final volume of 20 microliters, including 10 microliters of ready-to-use PCR reagents (Amplicon, Denmark), 1.5 microliters of each primer with a concentration of 0.5 picomolar, 2 microliters of DNA with a concentration of 100 ng and 5 microliters of deionized water. . In the first step of amplification, DNA synthesis was started with a hot start at 94°C for 2 minutes, and during 35 cycles with a thermal program of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. It continued for 30 seconds. At the end, the final multiplication stage was completed for 5 minutes at 72°C (Corbett thermocycler, model CG1-96, Australia) [9]. In order to confirm the initial amplification, from electrophoresis in 1.5% agarose gel in buffer (TBE) Tris base, boric acid, EDTA It was used with a voltage of 90 for 40 minutes. Next, in order to identify the lactic isolate, the PCR products after sequencing were aligned using the Basic local alignment search tool (Blast) procedure with the data available in the National Center for Biotechnology Information (NCBI) database.

2-5- Investigating the resistance of lactic isolate to acid and bile

For this purpose, the population of lactic isolates to CFU/mL 10^8 was set. Then, using 1 normal hydrochloric acid, the pH of the culture medium containing this bacterium was brought to about 2. In the next step, the mentioned bacterial suspension was placed in a hot house at a temperature of 37 degrees Celsius for 1.5 hours. Next, by using 1 normal sodium hydroxide, the pH of the said suspension was brought to about 6, and it was placed in a greenhouse in the vicinity of bile salt with a concentration of 0.3% at a temperature of 37 degrees Celsius for 1.5 hours. After successive acid and bile treatment, successive dilutions of the final suspension were prepared in sterile Ringer's and surface cultured on MRS agar culture medium and kept in a greenhouse at 37 degrees Celsius for 24 hours. Finally, live bacteria were counted compared to the control sample (without acid and bile treatment) [10].

6-2- Self-association and non-association capability of lactic isolate

To evaluate the self-adhesion property, the cells obtained from the 24-hour culture of the lactic isolate were separated by a refrigerated centrifuge (Hanil Kombay, South Korea) and washed with phosphate buffer in two steps. Then 4 ml of microbial suspension with a population of 10 CFU/mL^{8}It was kept in a greenhouse at a temperature of 37 degrees Celsius for 24 hours. Next, the optical absorption of this suspension was read at a wavelength of 600 nm and at zero and 24 hours. At the end, the amount of self-association ability of lactic isolate was calculated based on the following relationship. In this regard, A_0 Absorption amount at time zero and A_t The amount of absorption is after 24 hours [10].

$[1-(A_t/A_0)] \times 100$ = the amount of self-connection

To evaluate the binding property of lactic isolate with pathogenic bacteria (*Staphylococcus aureus* ،*Listeria monocytogenes* ،*Escherichia coli* And*Bacillus cereus*), cells obtained from 24-hour culture of lactic isolate were separated by refrigerated centrifuge and washed twice with phosphate buffer. Then, a mixture of equal volumes of the suspension of each pathogenic bacteria and the suspension of the lactic isolate was prepared and kept in a greenhouse at a temperature of 37 degrees Celsius for 4 hours. Next, the optical absorption of the mixed suspensions was read at a wavelength of 600 nm, and the amount of different binding property was calculated through the following equation. In this regard, A_{lac} Absorption of lactic isolate suspension, A_P attraction Suspension of pathogenic bacteria and A_{mix} Absorption of the

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suspension mixture of lactic isolates and pathogenic bacteria [11].

$[(A_P+A_{lac})/2 - (A_{mix})/(A_P+A_{lac})/2] \times 100$ = the amount of other connection

2-7- Antibacterial effect of lactic isolate

To evaluate the antibacterial effect of lactic isolate against bacteria*Staphylococcus aureus* ، *Listeria monocytogenes* ،*Escherichia coli* And*Bacillus cereus* A two-layer cultivation method was used. First, the lactic acid isolate was cultured in MRS broth culture medium and the foodborne bacteria were cultured in Brain Heart Infusion (BHI) broth culture medium at 37 degrees Celsius for 24 hours. Lactic isolate population to 10 CFU/mL^{8}was delivered Then, 5 microliters of the active culture of the lactic isolate was spotted in the center of the plate containing MRS agar medium and kept in a greenhouse at 37 degrees Celsius for 72 hours. Next, the suspension of each foodborne bacteria with a population of 10 CFU/mL^6 BHI agar culture medium was poured on the stained lactic isolate as a two-layer culture. After the coagulation of the second layer, the plates were kept in a greenhouse at 37 degrees Celsius for 24 hours. Finally, the diameter of the non-growth halo around the lactic isolate was determined using Image J software (version 1.42e) [11].

2-8- Antibiotic resistance of lactic isolates

To evaluate antibiotic resistance, first, 200 microliters of 24-hour culture of lactic isolate was added to 4 milliliters of 1% (semi-solid) MRS agar culture medium. Next, the mentioned mixture was poured on the surface of the plates containing MRS agar 1.5% solid. Then common antibiotic disks, including vancomycin, ceftriaxone, novobiocin, imipenem, nalidixic acid, clindamycin, ciprofloxacin, streptomycin, ampicillin, penicillin, cefazolin, cephalothin, kanamycin and gentamicin were placed on the surface of each plate. The plates were kept in a greenhouse at a temperature of 37 degrees Celsius for 24 hours. Finally, the diameter of the growth halo around the discs was measured by Image J software. The results were reported as resistant (diameter less than or equal to 14 mm), sensitive (diameter more than 20 mm) and relative sensitivity (diameter 15 to 19 mm) [12].

9-2- Ability of hemolysis of blood by lactic isolate

To check blood hemolysis ability, lactic isolate was cultured on the surface of agar culture medium containing 5% sheep blood and after 48 hours of greenhouse at 37 degrees Celsius, halo formation and color change in the culture medium were investigated [10]. .

2-10- Statistical analysis of the results

All the tests of this research were done in 3 repetitions and for the purpose of statistical analysis, completely random basic design with one-way analysis of variance was used. The obtained data were also analyzed by SPSS version 26 statistical software. Also, comparison of means using the least significant difference (LSD) test at the 0.05 level*P*< Done. Microsoft office Excel 2013 software was also used to draw diagrams.

3. Results and Discussion

3-1- Isolation and identification of dominant lactic acid bacteria

According to the results of reference tests, the amaranth flour used in this research had 15.69% protein, 7.17% fat, 12.39% moisture, 3.30% ash, and 61.45% total carbohydrates. After the amaranth sourdough pH reached about 4, the dominant lactic acid bacteria was isolated by the previously described method. Based on the results of biochemical and morphological tests, the dominant lactic isolate of amaranth sourdough was a gram-positive, catalase-negative, and shape-loving bacterium. Gel electrophoresis of PCR products also confirmed the specific amplification of the target sequence of 1500 bp in the genomic DNA of the lactic isolate (Figure 1). Finally, based on the sequencing results of PCR products and their alignment with the data available in the NCBI database of lactic isolates,*Lactobacillus brevis* SKA01 (98% similarity) was identified.

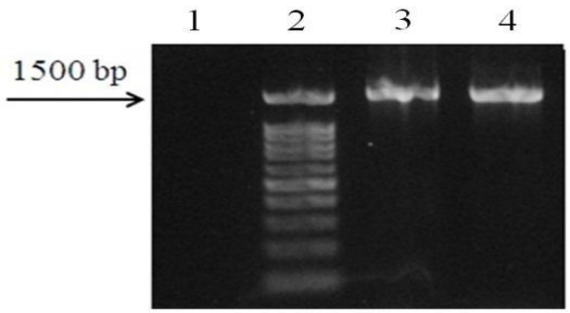

Fig 1 Gel electrophoresis of the PCR products. Lane 1: negative control, lane 2: DNA ladder, lane 3: amplified DNA of the LAB isolate and lane 4: positive control.

In the study of Cakir et al. (2020), after examining the microbial flora of fermented hulled barley,*Lactobacillus brevis* It was introduced as one of the dominant bacteria isolated from this fermentation medium [13]. Other researchers as well*Lactobacillus brevis* 173 were isolated from rye sourdough [14].

Ruiz Rodriguez et al. (2016) after random amaranth fermentation and inoculation process for 10 days, varieties of*Lactobacillus* ، *pediococcus* And*Enterococcus* were isolated from the aforementioned sourdough [8]. The internal factors of the substrate (carbohydrates, nitrogen sources, minerals, lipids and enzyme activity) and process components (dough yield, oxygen, fermentation time, fermentation temperature and number of fermentation processes) significantly affect the microbial flora of sourdough [15]. At the beginning of the fermentation process in the random fermentation of sourdough, we mainly encounter homofermentative lactobacilli, but later on, heterofermentative lactobacilli dominate. Three factors increase the competitiveness and compatibility of lactobacilli with the special environment of sourdough. These factors include their compatibility with the metabolism of carbohydrates as the main sources of energy in dough, matching the growth needs of lactobacilli to the temperature and pH of fermentation, having several response mechanisms to overcome the created stresses (such as acid production, osmotic conditions, oxidation and reduction of substances food) are In addition, the production of antimicrobial compounds, including organic acids (such as lactate and acetate) and antimicrobial metabolites such as bacteriocins, improves the competition of lactobacilli and can help their stability in sourdough [16].

2-3- Investigating the survival of lactic isolates in acid and bile

The survival of lactic isolates after sequential acid and bile treatment compared to the control sampleCFU/mL 10^8 to aboutCFU/mL 10^6 arrived and decreased by two logarithmic cycles. Fekri et al. (2020) Zindamani*Enterococcus faecium* ،*Pediococcus pentozaceus* And*Leuconostoc Citrum* isolated from Iranian traditional sourdough at pH equal to 2 and bile salts with a concentration of 0.3% were investigated for 3 hours.*Enterococcus Fascium* AR-L02 showed the highest level of resistance at low pH (95%) and in the presence of bile salts (79%) compared to strains*Pediococcus pentozaceus* And*Leuconostoc Citrum*showed [17]. During a similar survival study*Pediococcus pentozaceus* PRK1,*Lactobacillus plantarum* PRK7 and*Lactobacillus plantarum* PRK11 isolated from fermented rice bran was investigated. These isolates were resistant to pH = 2 and their survival in bile was 0.3%*Respectively* 88, 86.5 and 81.3 percent [18].

According to the research of Adesulu-Dahunsi et al. (2018), 5 lactic isolates from Nigerian fermented grain-based foods, including*Lactobacillus plantarum*YO175 ، *Lactobacillus plantarum*OF101 ،*Pediococcus pentozaceus*OF31 ،*Visula Confusa*OF126 And*Wisla Confucius*WS90 had good survival at pH = 2 and pH = 3. All isolates (except *Visula Confusa* WS90) showed good resistance against 0.3% bile salts for 4 hours [19]. According to the report of Sadeghi et al. (2019), among the 3 rice bran sourdough lactic isolates that were able to survive at pH equal to 2 and simulated conditions of the digestive system,*Lactobacillus brevis* had the longest survival [20]. The most important factors affecting the survival of probiotic bacteria in the digestive tract include the ability to tolerate low pH and hydrolysis of bile salts. Common mechanisms for acid resistance in lactic acid bacteria are proton pumping by the activity of F_1-F_0-ATPase, glutamate decarboxylase system, ammonia cloud formation, high urease activity, repair or

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protection of macromolecules and biofilm formation and neutralization processes [21]. Bile tolerance by probiotics is also related to the activity of their hydrolysis enzyme, which helps to hydrolyze conjugated bile and reduce its toxic effect [22].

3-3- Self-association and non-association capability of lactic isolate

In this study, the rate of self-adhesion of the lactic isolate reached 1% after 4 hours of incubation at 37 degrees Celsius and 36.19% after 24 hours. The rate of reconnection of the separation after 24 hours with*Escherichia coli*(61/0 ± 99/37) ،*Staphylococcus aureus*(23/0 ± 24/71) ،*Listeria monocytogenes* (24.18 ± 1.05) and*Bacillus cereus* (10.22 ± 0.70) percent which is significant (>0.05).*P*) differed from each other (Figure 2).

Self-association and other-association are important features related to the beneficial effects of probiotics, which are defined as the accumulation of bacteria of the same type and the accumulation of bacteria of different strains, respectively. Self-adhesion is the factor of settling and the necessity for the continued presence of probiotics in the digestive system, and the higher the percentage of self-adhesion, the greater the ability of probiotic bacteria to attach to epithelial cells. Dagher is considered a barrier to prevent the colonization of pathogenic microorganisms on the surface of the intestine [23,24].

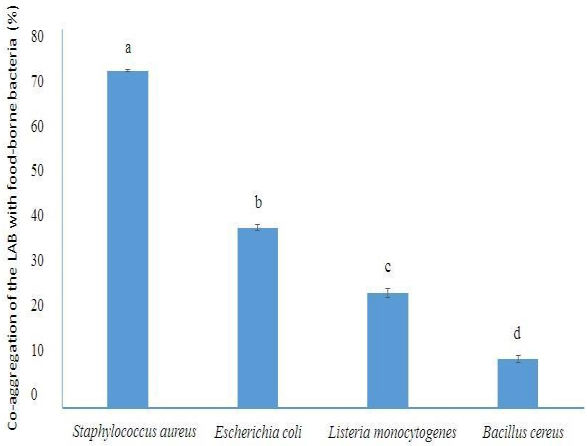
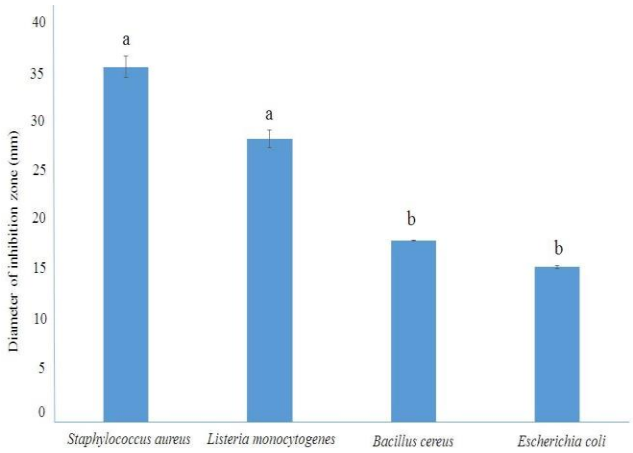


Fig 2 Co-aggregation percentage of the LAB isolate with food-borne bacteria. Different letters show significant difference at *P*<0.05.

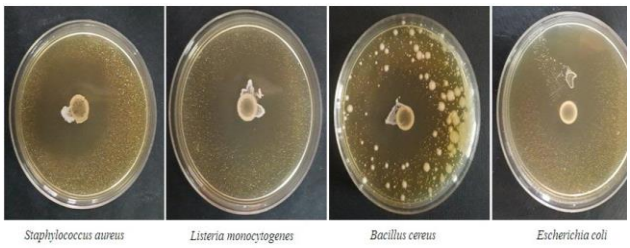
Vasiee et al. (2020) Self-association rate*Pediococcus pentozaceus* DHR005 28.8% and*Pediococcus lactic acid* They declared LAH-5 to be 48%, while the self-association rate of the lactic isolate reached 36.19% after 24 hours in the mentioned study [25]. Han et al. (2017) reported that among the 10 lactic acid bacteria investigated*Lactobacillus Bruce* It had the highest amount of self-adhesion. In this way, its self-adhesion rate reached about 2% after 4 hours of incubation at 37 degrees Celsius and 80% after 24 hours [26]. According to researchZangeneh et al. (2019) Self-joining and other-joining capability*Lactobacillus plantarum*KHMZ during 5 hours of incubation at room temperature significantly from*Lactobacillus plantarum*MZRF was more so that this strain has the highest accumulation with*Staphylococcus aureus*showed [27]. The different binding percentage of pathogens and probiotics depends on their strain and greenhouse time. The ability of commonly isolated lactic acid bacteria to bind to pathogenic agents may be attributed to their cellular components. Probably, interactions between carbohydrate-lectin and protein components on the cell surface are effective in this phenomenon [28,29].

3-4- Antibacterial effects of lactic isolate

As shown in Figure 3, the inhibitory effect of lactic isolate on*Staphylococcus aureus* And*Listeria monocytogenes* Than*Escherichia coli* And*Bacillus cereus* Significantly (>0.05).*P*) was more, but between the antibacterial effect of this isolate on*Staphylococcus aureus* And*Listeria monocytogenes*, significant difference (0.05*P*>) was not observed.



(A)



(B)

Fig 3 Diameter of inhibition zone of the food-borne bacteria in the present of the LAB isolate in antibacterial assay. The different letters show significant difference at $P<0.05$ (A), and co-culture of the LAB and each food-borne bacterium (B).

Somashekaraiah et al (2021) Antimicrobial effect*Lactobacillus brevis*MYSN105 isolated from the fermented product of Puja[1] against*Staphylococcus aureus* And*Escherichia coli* they studied These researchers, the diameter of the aura of lack of growth*Staphylococcus aureus* 21 mm and*Escherichia coli*RA They reported 18 mm. In the present study, the diameter of the lack of growth halo*Staphylococcus aureus* 35 mm and*Escherichia coli* It was 15 mm [30]. The results of two studies indicate that the antimicrobial effect*Lactobacillus brevis* SKA01 in the present study vs*Staphylococcus aureus*More and against *Escherichia coli* fewer Was.

In a research, Ramos et al. (2013) isolated 51 lactic acid bacteria with suitable viability and investigated their antibacterial effect, and among these isolates, 2 types*Lactobacillus brevis* SAU105 and FFC199 and*Lactobacillus fermentum* Has an inhibitory effect against*Listeria monocytogenes* And*Staphylococcus aureus* were [31]. According to the study of Tavakoli et al. (2017) all isolates except*Lactobacillus plantarum*MT.ZH293 and*Lactobacillus pentosus*MT.ZH693, growth*Staphylococcus aureus* And*Pseudomonas aeruginosa* inhibited [32].

Probiotics prevent the harmful effects of intestinal pathogens by several mechanisms, such as competition for nutrients, inhibiting the adhesion of pathogenic agents to mucosal cells, inhibiting the invasion of pathogenic agents to epithelial cells, producing antimicrobial metabolites, or stimulating mucosal immunity [33]. Antimicrobial metabolites of lactic acid bacteria include organic acids (lactic acid, acetic acid, formic acid, propionic acid and butyric acid) whose action is intensified by lowering the pH of the environment, as well as other metabolites such as ethanol, fatty acids, hydrogen peroxide, diacetyl, Low molecular weight compounds such as bacteriocins (nisin, reuterin, rheutericycline, pediocin, lacticin and enterocin) and bacteriocin-like inhibitory compounds [34]. Bacteriocins usually inhibit the growth of gram-positive pathogenic bacteria, and the main reason for inhibiting the growth of gram-negative pathogenic bacteria is the presence of hydroxy fatty acids, organic acids, and hydrogen peroxide. Therefore, in the case of lactobacilli, the main factor of antimicrobial activity is the production of bacteriocin, but the presence of compounds such as hydrogen peroxide, organic acids, ethanol and competition for nutrients are also effective in this feature [35,36].

5-3-antibiotic resistance of lactic isolate

Based on the antibiotic resistance results shown in Table 1, the lactic isolate is resistant to kanamycin, nalidixic acid, vancomycin, ciprofloxacin, streptomycin, ceftriaxone, clindamycin, novobiocin, gentamicin, and imipenem, and to ampicillin, cefazolin, and cephalothin. , had relative sensitivity and was also sensitive to penicillin.

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Table 1 Resistance of the LAB isolate towards some routine antibiotics determined based on the disc diffusion bioassay.

Zone of Inhibition (mm)	Susceptibility profile	Antibiotic (µg)
0.09^{d}± 6.37	resistant	Clindamycin (2)
$0.39^{It\ is}$± 3.45	resistant	Gentamicin (10)
0.00^{f}± 0.00	resistant	Streptomycin (10)
0.00^{f}± 0.00	resistant	Ciprofloxacin (5)
1.93^{a} ± 21.91	sensitive	Penicillin (10)
0.00^{f}± 0.00	resistant	Vancomycin (30)
$0.1^{It\ is}$ ± 3.26	resistant	Novobiocin (5)
0.28^{b} ± 15.94	semi resistant	Cephalothin (30)
0.79^{b} ± 14.85	semi resistant	Ampicillin (10)
0.05^{b} ± 14.52	semi resistant	Cefazolin (30)
0.42^{c} ± 10.13	resistant	Ceftriaxone (30)
0.00^{f}± 0.00	resistant	Nalidixic acid (30)
$0.19^{It\ is}$ ± 2.31	resistant	Imipenem (10)
0.00^{f}± 0.00	resistant	Kanamycin (30)

Different letters are significantly different ($P< 0.05$) within the column.

During the study of Sadeghi et al. (2019)*Lactobacillus brevis* isolated from rice bran sour dough, it was resistant to vancomycin, streptomycin and nalidixic acid antibiotics and sensitive to penicillin, which is similar to the results of the present study. Of course, the antibiotic ability of the isolates regarding the antibiotics nobiocin, ciprofloxacin, gentamicin, clindamycin and ceftriaxone was completely different in these two studies [20].

In research Vasiee et al. (2018) all isolates were vancomycin resistant. In the mentioned research, the aura of lack of growth compared to penicillin for*Lactobacillus brevis* B12 equivalent to 26.56 mm, strain*Lactobacillus brevis*B16, 18.67 mm, diameter*Lactobacillus Bruce* C33 was 17.85 mm [37] while in the present study, the aura of lack of growth*Lactobacillus Bruce* SKA01 compared to penicillin was 21.91. This isolate showed the highest sensitivity to penicillin among the tested antibiotics. Based on the results reported byMunoz et al. (2014),All isolates*Lactobacillus Pentosus* And*Lukonostok Pseudocentroides* They showed high sensitivity to amoxicillin, ampicillin, chloramphenicol, gentamicin and erythromycin. Also, these isolates were resistant to streptomycin and vancomycin [38].In researchThat et al. (2019) all Lactobacillus isolates studied were sensitive to ampicillin, erythromycin and clindamycin, while they showed resistance to streptomycin, kanamycin and ciprofloxacin. Also, most Lactobacillus species were resistant to gentamicin [39]. There is always the concern that lactic acid bacteria and bifidobacteria as part of the bacterial population of food and the digestive system of humans and animals can act as a reservoir of antibiotic resistance genes. This resistance can eventually be transferred to pathogenic and opportunistic bacteria and prevent the treatment of infections. Different mechanisms for resistance to antibiotics have been reported, but which mechanism is proposed in a specific bacterial strain depends on the nature of the antibiotic, its target site and the type of bacteria, which inhibits processes such as cell wall synthesis (the most common mechanism), protein synthesis, translation , cell membrane change, nucleic acid synthesis and metabolic activity [40]. In addition, antibiotic resistances in bacterial genera or species may be inherent or acquired through one or more successive mutations or by combining new genes. Antibiotic resistance genes can be transferred through transposable elements (plasmids and transposons) [41].

6-3- Blood hemolysis ability

In this research, the lactic isolate had hemolytic activity of gamma type.

In general, the hemolysis activity of microorganisms is divided into 3 categories: alpha, beta and gamma. Observing green color indicates alpha hemolysis and yellow or clear color indicates beta hemolysis. If no color change is observed around the isolate, it is gamma hemolysis.

During the research of Sharma et al. (2019), among the 26 lactic isolates of wheat flour sourdough, 20 cases were hemolysis negative and 6 cases were hemolysis positive [42]. Also, in another study by Argyri et al. (2013), among the 69 isolates studied, most of the isolates were gamma hemolysis and 4 strains*Lactobacillus pentosus*B278, B279, B281 and B285 had alpha hemolysis and none of the isolates had beta hemolysis [43]. In similar reviews*Lactobacillus*

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*Bruce*KT16-2 and*Lactobacillus brevis* S82 also lacked hemolytic activity [44,45].Lack of hemolytic activity to classify bacteria as (GRAS)Generally Recognized As Safe It is required according to the rules of the European Food Organization.

4- Summary

In this research, evaluation of characteristics*Lactobacillus brevis* As the dominant lactic isolate of fermented amaranth, it showed that this isolate had good probiotic capabilities. Due to the appropriate survival of this isolate in acidic conditions and the presence of bile salts, resistance to antibiotics, self-adhesion and anti-adhesion capabilities, suitable antibacterial effects of the isolate.*Lactobacillus brevis* Can that introduced as a potential candidate for use as a probiotic microbial culture in fermentation industries.

5-Resources

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ارزیابی ویژگی‌های پروبیوتیکی *لاکتوباسیلوس برویس* به عنوان باکتری اسید لاکتیک غالب جدا شده از آمارانت تخمیر شده

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چکیده

ارزیابی ویژگی‌های پروبیوتیکی باکتری‌های اسید لاکتیک جدا شده از شبه غلات تخمیر شده در تهیه کشت‌های میکروبی اهمیت وافری دارد. در پژوهش حاضر، پس از شناسایی مولکولی، خصوصیات پروبیوتیکی باکتری اسید لاکتیک غالب جدا شده از تخمیر آمارانت مورد ارزیابی قرار گرفت. توالی‌یابی محصولات PCR منجر به شناسایی *لاکتوباسیلوس برویس* به عنوان جدایه لاکتیکی غالب گردید. زنده‌مانی جدایه مذکور پس از تیمار متوالی اسید و صفرا در مقایسه با نمونه کنترل از 10^8 به حدود 10^6 CFU/mL رسید و بیشترین اثر ضد باکتریایی را در برابر *استافیلوکوکوس اورئوس* از خود نشان داد. با این‌حال، هاله عدم رشد *استافیلوکوکوس اورئوس* در حضور جدایه لاکتیکی در مقایسه با هاله عدم رشد *لیستریا مونوسیتوژنز* اختلاف معنی‌داری نداشت ($P>$ ۰/۰۵). همچنین جدایه لاکتیکی قابلیت خود اتصالی (۳۶/۱۹ درصد) و دگر اتصالی مناسبی با *استافیلوکوکوس اورئوس* (۷۱/۲۴ درصد) داشت و فاقد فعالیت همولیزی بود. علاوه بر این، نسبت به اکثر آنتی‌بیوتیک‌های مورد مطالعه مقاوم بود. با توجه با قابلیت‌های پروبیوتیکی مناسب *لاکتوباسیلوس برویس* جدا شده از تخمیر آمارانت می‌توان از آن به عنوان کشت میکروبی آغازگر و یا پروبیوتیک در صنایع تخمیری استفاده نمود.