Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

The viability of *Lactiplantibacillus pentosus* v390 under acidic and bile conditions, and evaluation of its antimicrobial activity and safety

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ARTICLE INFO

Article History:
Received:2024/3/4
Accepted:2024/4/9

Keywords:

Biogenic amines,

Cholesterol adsorption,

Antibacterial activity,

Hydrophobicity.

DOI: **10.22034/FSCT.21.153.192.**

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ABSTRACT

In this study, molecular identification of *Lactiplantibacillus pentosus* v390 was performed using 16S rRNA gene analysis with FYM27 and R1492 primers. The viability of the strain was assessed under acidic conditions at pH 2.5, 3.5 and 4.5, and resistance to bile at concentrations of 0%, 0.3%, 0.5%, and 0.7% was investigated. Antioxidant activity, cholesterol absorption, hydrophobicity, potential for DNase enzyme production, biogenic amines, hemolytic activity, and resistance to common therapeutic antibiotics were evaluated. The antimicrobial effect of the strain against pathogenic bacteria (*Shigella dysenteriae*, *Salmonella enterica serovar Typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus subtilis*) was examined using the well and disk diffusion method. The results showed that the *L. pentosus* v390 strain had the ability to survive at different pH levels, but after three hours of storage at pH 2.5, the bacterial count decreased. The strain demonstrated growth capability at various bile salt concentrations. *L. pentosus* v390 was intermediate to the antibiotic Nitrofurazone, resistant to Nalidixic and Imipenem antibiotics, and sensitive to Vancocin, Gentamicin, Chloramphenicol, Penicillin, and Ciprofloxacin antibiotics. The hydrophobicity, antioxidant activity (DPPH and ABTS), and cholesterol absorption of the strain were 46.50 ± 0.38%, 37.20 ± 0.40%, 39.90 ± 0.45%, and 36.50 ± 0.47%, respectively. No DNase enzyme production, biogenic amines, or hemolytic activity were observed from the strain. *L. pentosus* v390 exhibited stronger antimicrobial effects against Gram-positive bacteria. The results indicated that *L. pentosus* v390 has desirable probiotic properties that require further research to confirm its application potential in food product development.

[DOI: 10.22034/FSCT.21.153.192]

1- Introduction

A variety of microorganisms are presented under the heading of bacteria and probiotics. Lactic acid bacteria, in other words, a microorganism, probiotic, is based on the address of GRAS, an approved food and beverage supplier. He and Sazman of Aruba's food security. These microorganisms are used to create an effective Kurdish work in a person's home [1 & 2]. The drain of bacteria and lactic acid with a sufficient level (10^7 CFU/mL) is positive for the presence of a defect, a risk for the emergence of types of cancer and their tumors, strengthening the security system, suppression Cholesterol, lactose-digesting supplement, calcium supplement, and... [3 & 4]. Bacteria, which contain lactic acid, are a source of carbohydrates and produce compounds such as bacteriocins, diacetyl, acid, and hydrogen peroxide. This is evidence of its effectiveness against the microbe Bacteria pimarisa [5]. The mechanism of action of bacteria, such as lactic acid, has been mentioned as a means of controlling the nutritional factors and there is a connection, changing the matrix and other parameters, and moving the right-hand system, which can activate the logger to form a device in the device D [6]. *Lactiplantibacillus* is an important bacterium with lactic acid. You will stand. *Lactiplantibacillus* is a bacterium that has a stabilized, bioactive, non-sporing, catalase-free, bacteriostatic and microscopic properties [3]. Based on the pH level, the normal pH of *Lactiplantibacillus* is approximately 5.5 to 5.8 EST. When reading about the number of bacteria mentioned above, you can use a pH meter of 5 to reach the appropriate level. There is a high level of bacteria, such as lactic acid, which has the ability to grow at a different level (2-53 degrees Cantigrad) [7]. *Lactiplantibacillus* is found to contain a lot of evidence of the drug's clonogenic properties, as part of the properties of the herbal medicine, which is effective against this type of microbe. *Lactiplantibacillus* is in the mouth of a person's stomach, and 1 to 6 days of monitoring for microbial fluorescence in the body [4]. In order to know why this bacterium is within the capacity of your phone, you must wait a little while to make a mistake. Take the name of the device into your device. In terms of the probiotic properties of peptides, it has various osmone levels, such as tolerating acid and bile requirements, peptides, cholesterol scavengers, and tonic hydrolases High bile, non-hemolytic acid, pentansil, anti-microbial activity and... its benefits are not recommended [8].

The purpose of this study is to read about its probiotic properties, antimicrobial activity and the balance of bacterial security of *Lactiplantibacillus pentosus*. Based on the information available in this country, *Lactiplantibacillus pentosus* is known as *Lactobacillus pentosus* [9]. From a positive perspective, the bacteria are a source of caution, they have a zero resistance to acid and nickel, a sensitivity balance with a common antibiotic, a cholesterol balance, the amount of enzyme production. DNase and biogenic amino acids, combined absorption of antibiotics, antibiotics and activity against the bacteria *Lactiplantibacillus pentosus* v390 used.

2- Materials and methods

2-1- Isolation and identification of *L. pentosus* v390 strain

After collecting local yogurt samples randomly, they were transferred to the laboratory under refrigeration conditions. 45 mL of 0.1% peptone water was added to 5 grams of the samples. After the samples were homogenized by a homogenizer (Seaward, Germany) and serial dilutions of 10^{-1} -10^{-6} to were prepared, they were cultured on MRS Agar medium. The strain was isolated from the culture medium. Then gram staining and catalase test were

[DOI: 10.22034/FSCT.21.153.192]

performed. General primers FYM 27 (5′-AGA GTT TGATYMTGG CTC AG-3′) and R 1492 (5′-GGT TAC CTT GTT ACG ACT T-3′) were used in this study based on the amplification of the protected region of rRNA fragment S16 [10]. The results showed that the isolate with catalase-negative and Gram-positive properties with a similarity rate of 98% belongs to *L. pentosus* v390 strain.

2-2- Acid resistance test

In order to check the resistance to acid, after 18 to 24 hours of inoculation and keeping the bacteria in a greenhouse at a temperature of 37°C, bacterial cells were isolated from the culture medium using a refrigerated centrifuge model (Hermle, made in Germany). After separating the precipitate obtained using a spectrophotometer (Wp, Biowave II, made in England) with a wavelength of 600 nm, it was dissolved in a sterile phosphate buffer with an absorbance of 0.6. 50 microliters of the prepared microbial suspension were inoculated in 4 microtubes containing 450 microliters of sterile acidic phosphate buffer with different pHs of 2.5, 3.5, and 4.5 and placed in a greenhouse under anaerobic conditions and at a temperature of 37 degrees Celsius. After zero, 1, 2 and 3 hours of incubation of each of the samples, successive dilutions of sterile phosphate buffer up to 9-10 were prepared and surface culture was done on MRS Agar culture medium. With the completion of incubation for 24 hours at 37°C, the colonies formed on the surface of the culture medium were counted by the colony counter and the longevity of *L. pentosus* v390 bacteria was compared and evaluated with the control sample [11].

2-3- Bile resistance test

The level of resistance of *L. pentosus* v390 bacteria to bile salts was evaluated according to the method of Vasiee et al. (2018) [12]. After activating the bacteria, 100 microliters of the suspension were cultured on MRS Agar culture media containing zero, 0.3, 0.5 and 0.7% of bile salts. After finishing the greenhouse at 37°C for 48 hours, the results were observed visually.

2-4- Antibiotic sensitivity test

The sensitivity of the bacterial strain to common therapeutic antibiotics such as Vancocin, Gentamicin, Nitrofurazone, Chloramphenical, Nalidixic, Penicillin, Imipenem, Ciprofloxacin was evaluated. For this purpose, a suspension equivalent to half of McFarland's standard was prepared from the culture of *L. pentosus* v390 strain and it was surface cultured on MRS Agar medium. Antibiotic disks were placed on the culture medium with the help of sterile forceps. After the completion of incubation for 48 hours at 37°C, the diameter of the halo of non-growth around the antibiotic discs was evaluated and the results were reported as resistant, semi-sensitive and sensitive [13].

2-5- Cholesterol removal test

After inoculation, *L. pentosus* v390 bacteria was placed in MRS Broth culture medium with cholesterol stock solution (Sigma-Aldrich) and 0.3% bile salt at 37°C for 24 hours. The uninoculated MRS Broth culture medium was considered as the control sample. The inoculated medium was centrifuged at 6000 rpm at 4°C for 8 minutes. Then, 0.3 mL of 95% ethanol (Merck, Germany) and 2 mL of KOH (Merck, Germany) were added to 0.5 mL of the samples' supernatant. 5 mL of hexane (Merck, made in Germany) and 3 mL of distilled water were added to the samples. The mixture was kept for 15 minutes at room temperature in order to separate the phase. Absorbance of the inoculated and non-inoculated sample was read at a wavelength of 570 nm and the standard absorption curve of different concentrations of cholesterol (zero, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 µg/mL) was

[DOI: 10.22034/FSCT.21.153.192]

drawn. became [14]. Cholesterol absorption was obtained from equation (1):

equation (1): Cholesterol absorption percent = $\frac{C-T}{C} \times 100$

C: cholesterol concentration (µg/ml) in non-inoculated culture medium and T cholesterol concentration (µg/ml) in inoculated culture medium.

2-6- Cell surface hydrophobicity measurement test

In this method, the target bacteria was placed in a centrifuge with a rotation of 6000 g for 15 minutes. In order to reach the optical density of the strains at 600 nm to 0.6 to 0.7 (OD0), the strains were immersed in buffer. Then 3 ml of them were added to 1 ml of n-hexadecane (Merck, Germany) and kept at room temperature for 15 minutes. The test tube containing the strain was placed in the vortex for 3 minutes. After storage at room temperature, finally its aqueous phase absorption (OD) was measured through equation (2) [11].

equation (2): Hydrophobicity percent = $\left[\frac{OD_0-OD}{OD_0}\right] \times 100$

2-7- Hemolytic activity test

The amount of hemolytic activity of *L. pentosus* v390 bacteria was investigated after linear culture on blood agar culture medium with 7% volume-volume of sheep blood [10]. The inoculated medium was kept in a greenhouse at 37°C for 24 hours and the color changes were investigated. The formation of a clear halo, a green halo, or the absence of a halo around the colonies indicated β-hemolysis, α-hemolysis, and γ-hemolysis, respectively. In this test, *Staphylococcus aureus* and *Escherichia coli* bacteria were used as control samples for β-hemolysis and α-hemolysis.

2-8- DNase enzyme production evaluation test

This test was carried out according to the method of Vasiee et al. (2020) [14].

2-9- Biogenic amine (BA) production test

The ability to produce biogenic amines by *L. pentosus* v390 bacteria by decarboxylation of amino acids on the culture medium containing amino acid precursors such as L-histidine, monohydrochloride, tyrosine disodium salt, L-ornithine and L-lysine according to the method of Barzegar et al. (2021), was done [11]. Bacteria were inoculated once in the MRS Broth culture medium with the mentioned amino acid precursors and pyridoxal 5-phosphate and once again in the culture medium without amino acid precursors and containing purple bromocresol (Sigma-Aldrich). With the completion of incubation for 2 to 5 hours, the formation of purple color in the culture medium indicated the production of biogenic amines.

2-10- Antioxidant potential evaluation test

Evaluation of the antioxidant activity of *L. pentosus* v390 bacteria was carried out according to the method of Kim et al. (2022) [15]. After inoculation of the desired bacteria and two-step washing with sterile phosphate buffer, it was placed in a centrifuge. 0.2 mM DPPH solution (Sigma-Aldrich) was added to the sample and kept at room temperature for 30 minutes. 300 ml of sample and 600 ml of ABTS solution (Sigma-Aldrich) were kept at room temperature for 30 minutes. Absorbance was read at 517 nm for DPPH and 734 nm for ABTS. Ascorbic acid solution was used as a positive control. The percentage of inhibition of free radicals by the desired strain was calculated through equation (3):

equation (3): Inhibition of free radicals percent = $(1- A_{sample} / A) \times 100$

In this formula, Asample is the absorption rate of the tested sample, A is the absorption rate of the control sample.

2-11- Antimicrobial test

[DOI: 10.22034/FSCT.21.153.192]

In order to evaluate the antimicrobial activity of *L. pentosus* v390 bacteria against pathogenic bacteria, two methods of diffusion with the help of Well Diffusion Agar and Disk Diffusion Agar were used. In order to evaluate the antimicrobial activity of the bacterium *L. pentosus* v390 by the agar well method according to the method of Naushad et al. (2021), from 3 Gram-negative pathogenic strains such as *Shigella dysenteriae* PTCC 1188, *Salmonella enterica serovar Typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922 and 3 Gram-pathogenic strains Positive ones such as *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC19115 and *Bacillus subtilis* PTCC 1023 were used [3]. After activating the disease-causing strains, the suspension was prepared according to the standard of half McFarland and cultured on MHA culture medium (Merck, made in Germany). Wells with a diameter of 6 mm were created in the culture media using the end of a sterile pipette. The activated *L. pentosus* v390 bacteria in MRS Broth culture medium was placed in a centrifuge (Hermle, Germany) at 5000 rpm for 10 minutes at 25°C. In order to prepare the supernatant without solid cells, it was removed from the supernatant and passed through a syringe head filter with a diameter of 0.22 mm. 100 microliters of the supernatant without acidic and neutral cells was poured into the drilled wells. After finishing the greenhouse for 48 hours at 37°C, the diameter of the growth halo around the well was measured with a ruler and reported in mm.

According to the method of Alizadeh Behbahani et al. (2022), the antimicrobial activity of *L. pentosus* v390 bacteria was evaluated by diffusion method in agar with the help of disk against the aforementioned pathogenic bacteria [7]. The suspension prepared from pathogenic bacteria was cultured on MHA culture medium according to the standard of half McFarland. After immersing the paper discs with a diameter of 6 mm for 15 minutes in the cell-free supernatant prepared from *L. pentosus* v390 bacteria, they were placed on the MHA culture medium with the help of sterile forceps. After the end of incubation for 48 hours at 37°C, the lack of growth around the disc was measured with a ruler and reported in mm.

3- Results and discussion

In the present study, after isolation and molecular identification, the percentage of life of *L. pentosus* v390 bacteria was investigated at different pHs. The results of the viability of *L. pentosus* v390 at different pH are shown in Figure 1. The results showed that the mentioned bacteria had the ability to survive in different pHs, while with the passage of time, the survival of the desired bacteria decreased in different pHs. As the pH decreased, the bacterial viability decreased. So that the number of *L. pentosus* v390 at pH 2.5 after 3 hours of storage, decreased significantly and reached from 8.20 ± 0.16 Log CFU/mL to 6.90 ± 0.23 Log CFU/mL. which was the largest logarithmic decrease compared to other pHs.

[DOI: 10.22034/FSCT.21.153.192]

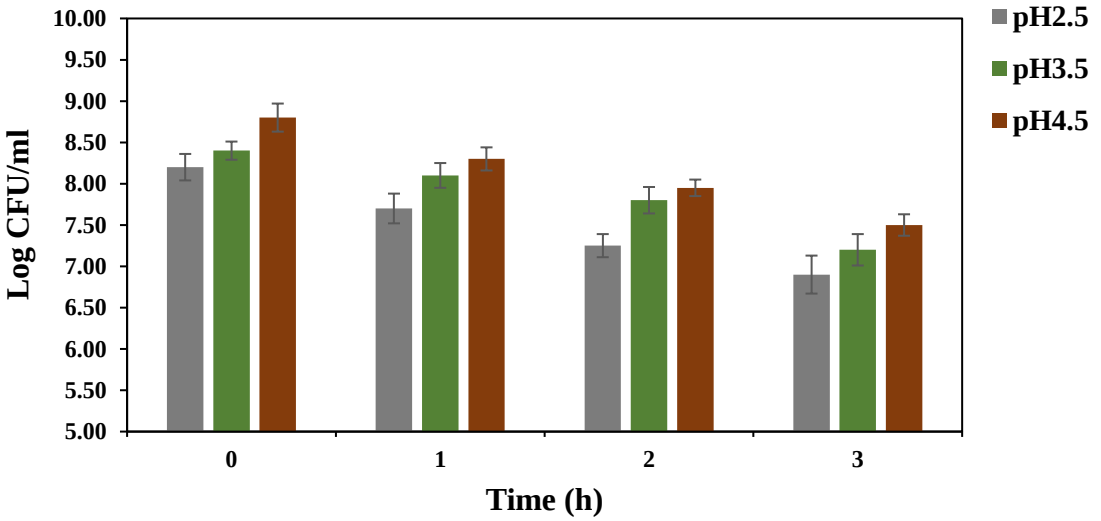

Fig. 1. The capacity of *Lactiplantibacillus pentosus* v390 to endure in an acidic pH environment.

In the present study, *L. pentosus* v390 bacteria had good resistance to different concentrations of bile salts, so that the bacteria in question maintained its ability to grow by increasing the concentration of bile salts up to 0.7%. The results of testing the resistance of *L. pentosus* v390 bacteria to different concentrations of bile salts are reported in Table 1.

Table 1. The ability *Lactiplantibacillus pentosus* v390 to endure varying concentrations of bile salts

Survivability	0.3%	0.5%	0.7%	Control
	Growth	Growth	Growth	Growth

In the present study, the immunity level of *L. pentosus* v390 bacteria was evaluated with tests to evaluate the level of hemolytic activity, the ability to produce DNase enzyme and biogenic amines. The results indicated that the bacterium in question lacked hemolytic activity, the ability to produce DNase enzyme, biogenic amines, and as a result, it was safe to produce probiotic food products. Also, the results of examining the sensitivity of *L. pentosus* v390 to common antibiotics showed that *L. pentosus* v390 was resistant to Nalidixic and Imipenem antibiotics, semi-sensitive to Nitrofurazone antibiotic and sensitive to other antibiotics (compared with CLSI table). The results of examining the sensitivity of *L. pentosus* v390 to different antibiotics are reported in Table 2.

Table 2. Effect of common therapeutic antibiotics on *Lactiplantibacillus pentosus* v390

[DOI: 10.22034/FSCT.21.153.192]

Antibiotic	*L. pentosus v390*
Nitrofurazone	**Intermediate**
Vancocin	**Sensitive**
Gentamicin	**Sensitive**
Chloramphenicol	**Sensitive**
Ciprofloxacin	**Sensitive**
Penicillin	**Sensitive**
Nalidixic	**Resistant**
Imipenem	**Resistant**

n the present study, the result of the cholesterol absorption test and the hydrophobicity of the cell surface by *L. pentosus* v390 were 36.50 ± 0.47 and 46.50 ± 0.38%, respectively. *L. pentosus* v390 had antioxidant activity (Figure 2). In the present study, the rate of inhibition of DPPH and ABTS free radicals by the strain was reported as 37.20 ± 0.40 and 39.90 ± 0.45%, respectively.

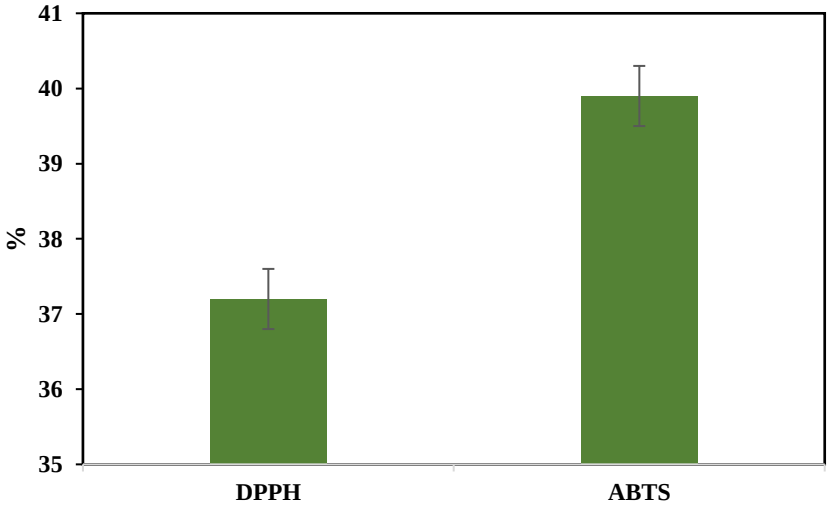

Fig. 2. The antioxidant activity (DPPH & ABTS) of *Lactiplantibacillus pentosus* v390

The results of evaluating the antimicrobial activity of the supernatant without acidic and non-acidic (neutral) cells of *L. pentosus* v390 on pathogenic bacteria by the diffusion method with the help of agar well and disk diffusion are reported in Figures 3 and 4, respectively. The results indicate that the strain had an acceptable inhibitory effect on pathogenic bacteria. Also, the supernatant without acidic cells had a greater antimicrobial effect on pathogens. Antimicrobial effect of the supernatant without neutral cells of *L. pentosus* v390 by diffusion method with the help of agar well on Gram-positive pathogenic bacteria *Staphylococcus aureus* with an inhibition zone diameter of 8.20 ± 0.20 mm, *Bacillus subtilis* with an inhibition zone diameter of

[DOI: 10.22034/FSCT.21.153.192]

8.10 ± 0.22 mm, *Listeria monocytogenes* with the diameter of inhibition zone was 7.90 ± 0.27 mm and Gram-negative bacteria *Salmonella enterica serovar Typhimurium* with the diameter of inhibition zone was 6.90 ± 0.24 mm. Antimicrobial effect of supernatant without acidic cells of *L. pentosus* v390 pathogenic bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Listeria monocytogenes*, *Salmonella enterica serovar Typhimurium*, *Escherichia coli* and *Shigella dysenteriae* with the diameter of inhibition zone 9.90 ± 0.35, 9.50 ± 0.20, 9.00 ± 0.46, 7.40 ± 0.29, 7.10 ± 0.13, and 7.00 ± 0.24 mm respectively. The most sensitive pathogenic bacterium against the antimicrobial effect of the supernatant lacking neutral and acidic cells was *Staphylococcus aureus*. Supernatant without neutral cells has antimicrobial effect on *Escherichia coli* and *Shigella dysenteriae* bacteria did not, while the supernatant without acidic cells had an antimicrobial effect on *Escherichia coli* and *Shigella dysenteriae* bacteria, which were the most resistant pathogenic bacteria against the antimicrobial effect of the supernatant without neutral and acidic cells (Figure 3).

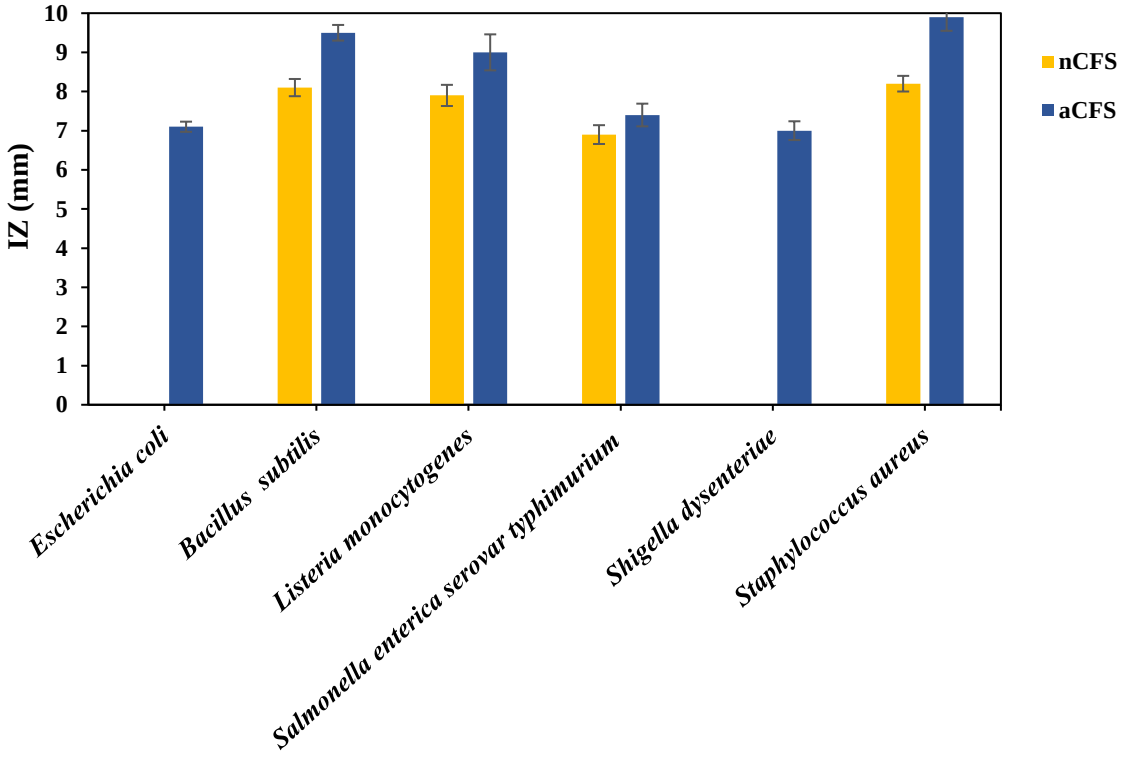

Fig. 3. The antimicrobial potency of *Lactiplantibacillus pentosus* v390 using well diffusion agar. The abbreviations aCFS and nCFS stand for acid cell-free supernatants and neutralized cell-free supernatants, respectively.

Antimicrobial effect of the supernatant without neutral cells of *L. pentosus* v390, by disc diffusion method, on Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Listeria monocytogenes*, respectively, with diameters of inhibition zone 8.10 ± 0.15, 7.70 ± 0.40, and 6.90 ± 0.32 mm. The diameter of the inhibition zone of pathogenic bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria*

[DOI: 10.22034/FSCT.21.153.192]

monocytogenes, *Salmonella enterica serovar typhimurium*, *Escherichia coli* and *Shigella dysenteriae* compared to the antimicrobial effect of the supernatant without acidic cells of *L. pentosus* v390 It was, 9.20 ± 0.25, 8.90 ± 0.18, 8.10 ± 0.27, 7.00 ± 0.20, 6.90 ± 0.17, and 6.80 ± 0.36 mm respectively. The supernatant without neutral *L. pentosus* v390 cells had no antimicrobial effect on *Escherichia coli*, *Salmonella enterica serovar typhimurium* and *Shigella dysenteriae* bacteria. As a result of the antimicrobial activity of the supernatant without acidic *L. pentosus* v390 cells in this way, it had an antimicrobial effect on all the mentioned pathogenic bacteria. The most sensitive pathogen bacteria against the effect of the antimicrobial activity of the supernatant lacking the desired neutral and acidic cell was *Staphylococcus aureus* and the most resistant pathogen bacteria against the effect of the antimicrobial activity of the supernatant lacking the desired neutral and acidic cell was *Shigella dysenteriae*, *Escherichia coli* and *Salmonella enterica serovar typhimurium*, respectively (Figure 4).

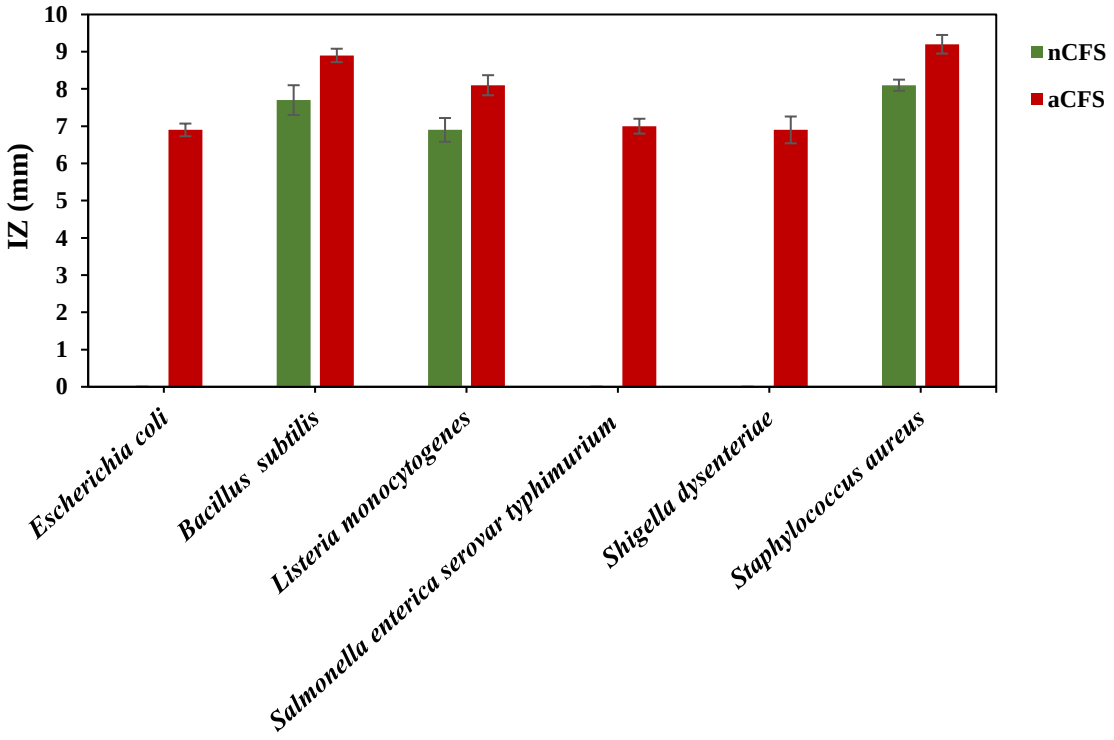

Fig. 4. The antimicrobial potency of *Lactiplantibacillus pentosus* v390 using disk diffusion agar. The abbreviations aCFS and nCFS stand for acid cell-free supernatants and neutralized cell-free supernatants, respectively.

By evaluating the resistance of probiotic bacteria to acidic conditions and the presence of bile salts, their survival rate can be checked [16]. In the present study, *L. pentosus* v390 strain had the ability to survive after 3 hours at pHs of 2.5, 3.5 and 4.5 and also the ability to grow at concentrations of 0.3, 0.5 and 0.7 bile salts. Based on studies in Gram-positive bacteria such as lactic acid bacteria, F1F0-ATPase combination makes them resistant and survive in acidic conditions [16]. Also, the presence of compounds such as polysaccharides protect lactic acid bacteria

[DOI: 10.22034/FSCT.21.153.192]

against stomach acid [17]. According to the research results, the average concentration of bile salt in the human digestive system is 0.3 percent by weight-volume. In order to reach the small intestine, probiotic microorganisms must have the ability to survive and pass through the stomach with a pH lower than 3 and the ability to pass through the duodenum with a bile salt content of up to 0.7% [6, 18]. On the other hand, it has been reported that lactic acid bacteria have the ability to convert bile salts into cholesterol and amino acids, so they can continue to survive and grow in the presence of bile salts [19]. Hosseini et al. (2018), viability of bacteria *L. plantarum* investigated microencapsulated in the simulated conditions of the stomach and intestine and reported that the survival rate of the bacteria after 2 hours was 2.03 ± 0.06 CFU/g [6]. Fatemizadeh et al. (2023), reported the survival rate of *Lactiplantibacillus plantarum* M17 in acidic conditions and bile salt concentration similar to human stomach as 91 and 89% respectively [20]. The aforementioned researchers reported these results as a confirmation of the appropriate resistance of the *L. plantarum* M17 strain to the conditions of the human digestive system. Vaseei et al. (2020), reported that the strain of *Pediococcus acidilactici* grew well in a concentration of 0.3% of bile salt, which was consistent with the results of the present study [14].

In the present study, the level of hydrophobicity of *L. pentosus* v390 strain was 46.50 ± 0.38%. The degree of hydrophobicity of the surfaces of lactic acid bacteria can influence their binding to intestinal epithelial cells. In some studies, it has been reported that the hydrophobic property of lactic acid bacteria is also effective in preventing colonization by pathogenic pathogens because pathogens continue to function by binding to intestinal cells, so lactic acid bacteria prevent the binding of pathogens by binding to intestinal cells [16]. Some compounds such as phosphate dehydrogenase, lipoteichoic acid, S-layer and lectin-like proteins play an effective role in binding lactic acid bacteria to intestinal cells [10]. In a study by Fallah et al. (2019), the level of hydrophobicity of *L. fermentum* strain 4-17 was reported as 43% [17]. In a research, the level of hydrophobicity of *L. acidophilus* strain B 14 was reported as 65.9% [11]. The reason for the difference in the amount of hydrophobicity of different probiotic strains can be the type of strain, the type of hydrophobic amino acids, the heterogeneity of the chemical structure, the proteins and lipids of the cytoplasmic membrane, the phase of cell growth, environmental factors and the degree of pleomorphism, van der Waals force, Brownian motion, surface electric charge and gravitational force. be related [3 & 11].

The results of the present study indicated that *L. pentosus* v390 bacteria was sensitive to Vancocin, Chloramphenicol, Ciprofloxacin, Gentamicin and Penicillin antibiotics, resistant to Nalidixic and Imipenem antibiotics and semi-sensitive to Nitrofurazone antibiotic. Lactic acid bacteria may be resistant to antibiotics. Antibiotic resistance may occur naturally in bacteria, while sometimes it may be caused by different genetic mechanisms, including gene transfer through plasmids, chromosomal mutations, etc. [21 & 22]. Other factors play a role in the resistance of lactic acid bacteria to antibiotics, such as the absence of the target site of the antibiotic in the probiotic bacteria, low permeability, inactivity of the antibiotic, etc. [22]. In a study, different *Lactobacillus* strains were isolated from fermented foods and their resistance to twelve antibiotics was investigated. In this study, the isolated strains were reported to be sensitive to the antibiotics Amikacin, Ampiclox, Ciprofloxacin, Clarithromycin, Cefotaxime, Levofloxacin, Cefuroxime, Cefoperazone, Gentamycin, Roxithromycin, Cotrimoxazole and Azithromycin [23]. Fatemizadeh et al. (2023), reported that *L. plantarum* M17 strain was sensitive to Ampicillin,

[DOI: 10.22034/FSCT.21.153.192]

Erythromycine and Chloramphenicol antibiotics and resistant to Kanamycine and Clindamycine [20].

In the present study, the cholesterol absorption rate of *L. pentosus* v390 strain was 36.50 ± 0.47%. Lactic acid bacteria have the ability to absorb cholesterol and remove hydroxyl radicals. The amount of cholesterol absorption by probiotics is related to the type of strain, the amount of production of compounds that lead to the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A enzyme [14].

In the present study, hemolytic activity and DNase enzyme production and the creation of a clear border around the colonies in blood agar culture medium were not observed from *L. pentosus* v390 strain. Also, no potential in the production of biogenic amines was observed in *L. pentosus* v390 strain. Therefore, this strain has a safe use in order to produce probiotic food products. Due to the fact that in the case of using probiotic bacteria in food products, it is very important not to hydrolyze essential amino acids, to evaluate the level of safety of the use and consumption of probiotic strains in food products from tests to evaluate the hemolytic property and the amount of DNase or deoxyribonuclease enzyme production that causes hydrolysis Phosphodiester bonds are used in the structure of DNA [20]. Lactic acid bacteria, especially *Enterococcus* and *Lactobacillus*, have the ability to produce undesirable metabolites such as biogenic amines, which are produced in food products containing protein and free amino acids that have undergone biochemical processes such as fermentation [11]. Fatemizadeh et al. (2023), reported that *L. plantarum* M17 bacteria lack the potential to produce biogenic amines [20]. In research, Alizadeh Behbahani et al. (2023), reported that *Limosilactobacillus fermentum* IMAU70160 bacteria isolated from local yogurt lacked DNase enzyme production, biogenic amines and hemolytic activity [16]. The results of the present study were consistent with the results of the aforementioned researchers.

In order to evaluate the antioxidant activity of *L. pentosus* v390 strain, DPPH and ABTS free radical inhibition tests were used. Inhibition of DPPH and ABTS free radicals by the desired strain was 37.20 ± 0.40 and 39.90 ± 0.45%, respectively. Lactic acid bacteria with iron and copper ions, organic acids, bacteriocins, bacterial exopolysaccharides, manganese, glutathione (GSH), butyrate, folate and superoxide dismutase enzyme have antioxidant activity, that is why they are considered as antioxidants with the ability to reduce oxidative stress in Food products are used [24 & 25].

One of the criteria for choosing lactic acid bacteria is their antimicrobial activity against pathogenic pathogens. In the present study, the results of the evaluation of antimicrobial activity indicated that the supernatant without acidic cells of *L. pentosus* v390 had a greater antimicrobial effect on pathogenic bacteria. Lactic acid bacteria due to competition with pathogens for food and the site of attachment to intestinal epithelial cells, as well as the production of compounds such as organic acids (acetic acid, lactic acid, propionic acid, sorbic acid, etc.), diacetyl, ethanol, hydrogen peroxide, Ammonia and low molecular weight proteins known as bacteriocins have antimicrobial activity [25 & 26]. In the present study, *L. pentosus* v390 strain had a greater antimicrobial effect on Gram-positive pathogenic bacteria. The mechanism of antimicrobial activity of lactic acid bacteria on Gram-negative and Gram-positive bacteria is different. According to research, lactic acid bacteria inhibit the growth and death of Gram-negative bacteria due to their ability to produce hydrogen peroxide, carbon dioxide and various organic acids. On the other hand, it has been reported that the production of bacteriocin by them leads to the destruction of Gram-positive bacteria [27-35]. In a study, the antimicrobial effect of *L. plantarum* strain M17 on *Cronobacter*

[DOI: 10.22034/FSCT.21.153.192]

sakazakii bacteria was reported [20]. In a study by Alburikan and Alshahrani (2024), the antimicrobial effect of different strains of *Lactiplantibacillus* was investigated. They reported that different strains had the ability to inhibit the growth of *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes* pathogens. In this study, the highest rate of inhibition of the growth of pathogens was related to the supernatant without acidic cells, and it was reported that the probiotic bacteria *L. pentosus* inhibited the growth of the mentioned pathogens by producing bacteriocin called Pentocin MQ1 [5].

4- Conclusion

Based on the results obtained from the current research, *L. pentosus* v390 bacteria had the ability to tolerate and survive in different acidic pH and different concentrations of bile salts. *L. pentosus* v390 had hydrophobic properties, antioxidant activity, cholesterol absorption ability and favorable antimicrobial potential against various pathogenic pathogens, no production of undesirable metabolites such as DNase enzyme, biogenic amines and no hemolytic activity. The desired probiotic bacteria showed sensitivity to most of the tested common antibiotics. Therefore, by confirming the probiotic, antimicrobial, and immune properties of *L. pentosus* v390 bacteria, it is necessary to conduct more studies in this field in order to more closely examine the functional characteristics and produce probiotic food products.

5- Acknowledgements

The authors would like to express their sincere gratitude to the Vice-chancellor for Research and Technology of Agricultural Sciences and Natural Resources University of Khuzestan for supporting this study as a project number 1402.40.

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[DOI: 10.22034/FSCT.21.153.192]

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[DOI: 10.22034/FSCT.21.153.192]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

قابلیت زنده‌مانی *Lactiplantibacillus pentosus* v390 در شرایط اسیدی و صفراوی، و ارزیابی فعالیت ضدمیکروبی و ایمنی آن

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۱۲/۱۴

تاریخ پذیرش: ۱۴۰۳/۱/۲۱

کلمات کلیدی:
آمین‌های بیوژنیک،
جذب کلسترول،
فعالیت ضدباکتریایی،
هیدروفوبیسیتی

DOI:10.22034/FSCT.21.153.192.

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چکیده

در این پژوهش، شناسایی مولکولی *Lactiplantibacillus pentosus* v390 توسط تجزیه و تحلیل ژن S rRNA ۱۶ به‌کمک پرایمرهای ۲۷FYM و ۱۴۹۲R انجام شد. قابلیت زنده مانی سویه در شرایط اسیدی در pHهای ۲/۵، ۳/۵ و ۴/۵، و مقاومت به صفرا در غلظت های صفر، ۰/۳، ۰/۵ و ۰/۷ درصد بررسی شد. فعالیت آنتی‌اکسیدانی، جذب کلسترول، هیدروفوبیسیتی، پتانسیل تولید آنزیم DNase و آمین‌های بیوژنیک، فعالیت همولیتیک، و مقاومت به آنتی‌بیوتیک‌های رایج درمانی ارزیابی شد. اثر ضدمیکروبی سویه علیه باکتری‌های بیماری‌زا (*Shigella dysenteriae*، *Salmonella enterica serovar Typhimurium*، *Escherichia coli*، *Staphylococcus aureus*، *Listeria monocytogenes* و *Bacillus subtilis*) به روش انتشار در آگار به کمک چاهک و دیسک بررسی گردید. نتایج نشان داد سویه *L. pentosus* v390 توانایی زنده‌مانی در pHهای مختلف را داشت اما با گذشت سه ساعت از نگهداری در pH ۲/۵ تعداد باکتری کاهش یافت. سویه مذکور قابلیت رشد در غلظت‌های مختلف نمک صفراوی را از خود نشان داد. *L. pentosus* v390 نسبت به آنتی‌بیوتیک Nitrofurazone نیمه‌حساس، نسبت به آنتی‌بیوتیک‌های Nalidixic و Imipenem مقاوم و نسبت به آنتی‌بیوتیک‌های Vancocin، Gentamicin، Chloramphenical، Penicillin و Ciprofloxacin حساس بود. هیدروفوبیسیتی، فعالیت آنتی‌اکسیدانی (DPPH و ABTS)و میزان جذب کلسترول سویه به‌ترتیب ۰/۳۸ ± ۴۶/۵۰، ۰/۴۰ ± ۳۷/۲۰، ۰/۴۵ ± ۳۹/۹۰ و ۰/۴۷ ± ۳۶/۵۰ درصد بود. تولید آنزیم DNase، آمین‌های بیوژنیک و فعالیت همولیتیک از سویه مشاهده نشد. *L. pentosus* v390 دارای اثر ضدمیکروبی بیشتری بر باکتری‌های گرم مثبت بود. نتایج نشان داد که *L. pentosus* v390 دارای ویژگی‌های پروبیوتیکی مطلوبی است که پژوهش‌های بیشتر به منظور تأیید قابلیت کاربرد آن در تولید محصولات غذایی ضروری است.

[DOI: 10.22034/FSCT.21.153.192]