Isolation of Probiotic Lactobacilli Bacteria from Traditional Naein Dairy Product (Koome)

Nina Shemshad 1, Leila Roozbeh Nasiraie 2,3*, Reza Majidzadeh Heravi 4

1. PhD Student, Department of Science and Food Technology, Nour branch, Islamic Azad University, Nour, Iran
2. Assistant Professor, Department of Science and Food Technology, Nour branch, Islamic Azad University, Nour, Iran
3. Manager of Research and development center, Shams Bavaran Salamat Nour Consulting & Production Services, Tehran, Iran
4. Assistant Professor, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

ABSTRACT

Background and Aim: Koome as one of the traditional fermented dairy products of ovine milk has long been produced in rural areas around Naein, Iran in sheepskin bags. The present study aimed to isolate Lactobacillus bacteria from the traditional dairy products of Naein and to evaluate the functional characteristics and health of these bacteria as probiotics.

Materials and Methods: For the initial isolation of bacteria, de Man, Rogosa, and Sharpe (MRS) agar was used. A total of 15 bacilliform, gram-positive, and catalase-negative colonies were isolated from the culture, and resistance to acid, bile, gastric juice, and intestinal juice was assessed to investigate probiotic characteristics. Bacterial isolates with favorable probiotic characteristics were tested for antimicrobial activity and antibiotic resistance to assess the effect of probiotics on health. Afterwards, seven bacterial isolates were selected and their ability for reducing cholesterol and hydrolyzing bile salts was evaluated. Moreover, the selected isolates were sequenced to identify the strain.

Results: Our findings demonstrated that six of 15 bacterial isolates had a suitable resistance in pH=2.5. In addition, 60% of the isolates were sensitive to bile salts. The identified Lactobacillus isolates had a high antibiotic resistance and were shown to have a favorable antimicrobial activity against pathogenic bacteria. Furthermore, the selected bacterial isolate could reduce 70% of environmental cholesterol.

Conclusion: According to the results of the present study, koome is highly potential for isolating probiotic isolates and the nutritional consumption of Lactobacillus isolates microbial supplement might have positive effects on health.

Keywords: Dairy products, Koome, Lactobacillus, Probiotic

Introduction

Fermented products, such as yogurt, kefir, sauerkraut, kombucha, and other dairy products, which have traditionally been used by people since ancient times now entered the field of biotechnology (1). Science of probiotic therapy as the result of the development of this process in food microbiology addresses the beneficial influences of probiotics (live microorganisms in food) in the host body (2).
Therapeutic effects and the positive impacts of probiotics on health are important due to stimulating the growth of intestinal beneficial microorganisms, decreasing the population of harmful bacteria, and helping the natural defense mechanisms of the body (3). Benefits of the lactic acid bacteria (LAB) isolated from traditional dairy products in preventing and treating diseases have been confirmed and no negative side effects have been noted for these probiotics (4).

Probiotic bacteria of the LAB group are gram-positive bacteria present in the microbial flora of the human digestive system. These bacteria are applied in food fermentation procedures and nowadays are considered as a mucosal barrier with the ability for regulating immune responses (5). According to the National Food Standard, probiotic bacteria should survive not only during the shelf life of food but also after passing through gastric acid, enzymes, and bile alkaline salts and should reach their activity site (intestine). Therefore, the foods which are claimed to impose healthy effects need to contain $10^7$ living probiotics per one gram at the time of consumption (1).

Lactobacilli were isolated from milk the first time and nowadays in the food industry, probiotics are known as a part of fermented dairy products, including kefir and soured milk. Over 70 products containing LAB are being produced throughout the world, namely sour cream, powdered milk, and fermented beverages (1). Koome is a traditional dairy product of ovine milk, which has long been produced in sheepskin bags in the rural areas around Naein, Isfahan province, Iran. Considering the unique physicochemical and microbiologic characteristics and the lack of salt, koome could be proposed as one of the best traditional dairy products with long shelf life.

Sharifi et al. (2017) evaluated 96 samples of traditional bovine, ovine, and caprine yogurt. Their results revealed that 47 samples had LAB with probiotic characteristics, including Lactobacillus lactis, L. brevis, and L. fermentum (6). Famouri et al. (2017) investigated the therapeutic characteristics and health effects of L. plantarum and L. brevis isolated from ten specimens of traditional fermented dairy products. Reduced serum cholesterol and heavy metals were among the favorable findings (7).

Handa et al. (2016) studied the LAB from two samples of fermented grain-based drinks. They isolated and identified the LAB with probiotic characteristics, such as Lactobacillus fermentum and Lactobacillus acidophilus and confirmed the positive effects of these bacteria on health (2). The present study aimed to isolate Lactobacilli with probiotic potential from the traditional dairy products of Naein, Iran. This genus of bacteria is widely used in diverse industries, namely the food, pharmaceutical, and supplements industries. Therefore, the recognition and classification of this genus of bacteria provide valuable information for researchers in different fields.

**Materials and Methods**

**Sampling and Microbial Culture**

A total of five bovine koome samples and three ovine koome specimens were collected from the surrounding rural areas of Naein. Next, for isolation, a homogeneity of 10 g of each sample in 90 mL of diluent was made and serial dilutions were obtained. The dilutions were inoculated on MRS agar and were incubated at 37°C for 24 h. The grown colonies were tested for isolating Lactobacilli from other organisms and morphologic evaluation. To this aim, each colony was cultured on medium to reach a single colony and purify. Afterwards, the purified culture was stored for further tests (8).

**Isolating Lactobacilli**

A catalase test was carried out to check the production of catalase enzyme by bacteria. In this test, a small part of the intended colony was placed on a sterile slide using a sterile loop and was mixed with a drop of hydrogen peroxide 3% (catalase reagent). The lack of bubble production means that the tested bacterium does not produce catalase enzyme and is known as catalase-negative. At the end of this step, the bacilliform, catalase-negative, and gram positive Lactobacilli colonies with different morphologies were coded and assessed for probiotic properties. Superficial culture was performed from the coded samples (9).

**Probiotic Evaluation**

**Resistance to Bile and Acid Conditions**

Survival of microorganisms was investigated in broth medium with acidic pH of 2, 2.5, and 3 similar to the digestive system. The number of living microorganisms was counted as presented as a percentage of the initial number following incubation at 37°C for 1 and 2 h. Resistance and growth reduction of microorganisms were assessed through incubating at 37°C for 8 h the presence of 0.7% and 1% of bile salts (bile oxalate).

The resistance of microorganisms in gastric and intestinal conditions was tested by inoculation to simulated broth media for the stomach (6.23 g sodium chloride, 0.229g calcium chloride, 2.29 g potassium chloride, 1.2 g sodium bicarbonate, pepsin enzyme with the concentration of 0.3%, and pH=2±0.2) and intestine (1.28 g sodium chloride, 0.239 g potassium chloride, 6.4 g calcium bicarbonate, 0.5% X-gal and...
pancreatrin enzyme with the final concentration of 0.1%, and pH=8). Next, culture was completed on agar medium and colonies were counted following overnight incubation at 37°C (8).

Identification of Acid-producing Bacteria

Acid production in culture medium by microorganisms was investigated based on pH reduction in the medium after 24 h incubation at 37°C. Bacteria with a lower pH than the initial pH of 6.22 were considered as acid-producing microorganisms (10).

Antimicrobial Activity

The ability of the intended isolates for producing antimicrobial compounds against standard pathogen bacteria was examined based on the presence of the zone of inhibition on agar medium. In this method, 200 µL of the active culture of pathogen bacterium was inoculated to a tube containing nutrient agar culture medium 1% (soft agar) and was added to nutrient agar 1.5% in a plate after cooling and was refrigerated for 30 min. Afterwards, sterile blank discs dipped in the supernatant of the isolate were fixed smoothly on the plate and were refrigerated for 20 min followed by incubation at 37°C. After incubation, the zone of inhibition was measured using a ruler and the presence of this zone was reported as antimicrobial impact against pathogen microorganisms (11).

Antibiotic Resistance

Sensitivity or resistance of probiotic bacteria to common antibiotics in medicine was evaluated by measuring the diameter of the zone of inhibition. First, active culture was prepared from probiotic bacteria. Next, 4 mL of sterile MRS agar 1% was poured into each tube. Following the cooling of culture media, 200 µL of fresh active probiotic culture was inoculated to each tube and was mixed thoroughly.

Plates containing MRS agar 1.5% were prepared and located at room temperature for 10 min to reach room temperature. Afterwards, a culture medium containing the prepared bacterium was gently added to the plate and refrigerated for 30 min until the bacteria were absorbed on the medium. Plates were taken out of the refrigerator and sterile antibiotic discs with a diameter of 0.7 cm were located and the plates were refrigerated for 20 min. Next, plates were incubated at 30°C for 12 h and the diameter of the zone of inhibition was measured on 8-12 h and the final diameter was presented in mm. Test results were reported as resistant, semi-sensitive, and sensitive according to the size of the zones of inhibition (12).

Cholesterol Reduction Test

In order to evaluate the ability of microorganisms for cholesterol hydrolysis, 0.2 mL of microorganism suspension in broth medium was inoculated to 20 mL of culture medium containing 100 µg/mL of cholesterol oxalate and was incubated at 37°C for 16 h. Afterwards, the tubes were centrifuged at 8000 rpm for 5 min at room temperature. Next, 0.5 mL of the supernatant was transferred to a glass tube and was mixed with 3 mL ethanol 95% followed by adding 2 mL potassium hydroxide 50%. The mixture was homogenized by 1 min vortex after the addition of each component.

The tubes were heated in a water bath of 60°C for 10 min and were cooled at room temperature. In the next step, 5 mL hexane was added to each tube and vortex was used for 20 sec followed by adding 3 mL of distilled water and 1 min vortex. The tubes were left at room temperature for 15 min or until the water and organic phases were completely separated. Afterwards, 2.5 mL of hexane layer (the upper layer) was poured into clean tubes and hexane was evaporated at 65°C in a water bath.

The liquid remaining in the tubes was mixed with 4 mL of o-phthalaldehyde and was kept at room temperature for 10 min. Next, 2 mL of sulfuric acid was added to each tube and was left at room temperature for 10 min. The absorbance of samples was read using a spectrophotometer at the wavelength of 550 nm versus blank (13).

Bile Salt Hydrolase Activity Assay

The zone of deoxycholic acid precipitation around the colonies in the culture medium containing the salt of bile acids was evaluated. To this aim, 10 µL of microorganism suspension was cultured on the surface of the MRX agar plate and was incubated at a suitable temperature. In the case of hydrolase activity, white precipitation and scattered zones surrounding colonies were clear. When these zones could not be observed, 0.037% calcium chloride could be added to the culture and blank discs dipped in 10 µL bacterial suspension are applied on the plate surface. A zone of white precipitation around the disc indicates bile salt hydrolysis by the tested bacterium (14).

Identification of the Isolates Selected by Probiotic Tests

Seven isolates with relative priority to other isolates in probiotic tests were identified by the DNA sequencing of the 16s ribosomal region. The mentioned region was amplified by polymerase chain reaction (PCR) utilizing Gradient Palm-Cycler (Corbett Life Science Pty. Ltd., Australia). General primers with the forward sequence of 5’GAG AGT TTG ATC CTG GCT CAG 3’ and the reverse sequence of 5’GAA AGG AGG TGA TCC AGC CG 3’ were applied for amplifying the intended segment (15).

The reaction set was as follow: 2 min at 95°C, 35 cycles at 95°C for 45 sec, 45 sec at 53°C, 60 sec at 72°C.
and the final step of 3 min at 73°C. The reaction product was electrophoresed on 0.8% agarose gel, the segment was extracted from the gel and after confirming the band length and concentration determination, it was sent to Microsynth, Switzerland for sequencing.

**Statistical Analysis**

The obtained data were analyzed as a random design with 3 repeats using SAS version 9.2. Moreover, Excel software version 2010 was used to draw the graphs.

**Results**

**Resistance to Bile Salts and Acid**

Findings of catalase test, gram staining, and the microscopic examination of isolates revealed that 15 colonies were bacilliform, gram positive, and catalase-negative, which were selected for probiotic tests and were encoded as S1-S15. The results of bile salts resistance assay at the concentrations of 0.3%, 0.7%, and 1% following 8 h of incubation are demonstrated in Table 1.

Moreover, the findings of testing X-gal 0.3% for the 15 intended isolates showed that S5 was highly resistant and S8, S9, S11, and S14 were resistant. In addition, S1, S2, S3, S4, S6, S7, S10, S13, and S15 isolates were sensitive. In media containing 0.7% and 1% bile salts. The isolates S8, S5, and S11 were resistant, while S9 and S14 were found as sensitive. As a result, 20% of the isolates were resistant to 0.7% and 1% concentrations of bile salts and S8, S5, and S11 were known as bile-resistant isolates.

Test of resistance to acid revealed that S15, S14, S13, S10, S2, and S1 were not sufficiently resistant to pH=3 following an hour of incubation at 37°C and had the viability percentage of zero. On the other hand, S11 and S12 had the highest viability rate of 96% followed by the isolates S9, S8, S7, S6, S5, S4, and S3 with the viability of 55%-60% after an hour of incubation. The resistant isolates in the latter step were tested at pH=2.5. The lowest and highest viability percentages following two hours of incubation at pH=2.5 were observed for S12 and S9, respectively. Afterwards, the resistant isolates in this stage were tested at pH=2. The results indicated that the most resistant bacteria to acid pH were S11, S7, and S5.

| Bacterium code | Inhibition coefficient 0.3% | Final result | Inhibition coefficient 0.7% | Final result | Inhibition coefficient 1% | Final result |
|---------------|-----------------------------|--------------|-----------------------------|--------------|---------------------------|--------------|
| S1            | 1                           | Sensitive    | -                           | -            | -                         | -            |
| S2            | 0.91                        | Sensitive    | -                           | -            | -                         | -            |
| S3            | 1                           | Sensitive    | -                           | -            | -                         | -            |
| S4            | 1                           | Sensitive    | -                           | -            | -                         | -            |
| S5            | 0.19                        | Highly resistant | 0.39              | Resistant    | 0.14                      | Resistant    |
| S6            | 1                           | Sensitive    | -                           | -            | -                         | -            |
| S7            | 0.84                        | Sensitive    | -                           | -            | -                         | -            |
| S8            | 0.31                        | Resistant    | 0.29                        | Resistant    | 0.28                      | Resistant    |
| S9            | 0.37                        | Resistant    | 0.98                        | Sensitive    | 0.74                      | Sensitive    |
| S10           | 1                           | Sensitive    | -                           | -            | -                         | -            |
| S11           | 0.43                        | Resistant    | 0.24                        | Resistant    | 0.43                      | Resistant    |
| S12           | 0.85                        | Sensitive    | -                           | -            | -                         | -            |
| S13           | 0.53                        | Sensitive    | -                           | -            | -                         | -            |
| S14           | 0.24                        | Resistant    | 0.62                        | Sensitive    | 1                         | Sensitive    |
| S15           | 1                           | Sensitive    | -                           | -            | -                         | -            |
Resistance to gastric juice was evaluated in 0, 30, 60, 90, and 120 min (Figure 1A). In this assay, isolates 5, 7, and 11 were tested as the isolates selected by acid test and 5, 8, and 11 as the isolates chosen by the bile resistance test. However, S14 was examined due to resistance to 0.3% bile and isolates 3, 4, 6, 9, and 12 were assessed because of resistance to pH=3.

The results are summarized in Figure 1. As could be observed, isolates 4, 14, and 6 had the lowest resistance to the simulated conditions of the stomach as viability reached zero after 30 min. isolates 8, 9, and 12, which were resistant to pH=2.5 but sensitive to pH=2, were destroyed after 120 min of exposure to simulated gastric conditions. The isolates resistant to pH=2, including 7, 11, and 5 were the most resistant bacteria to gastric simulated conditions following 2h of incubation. However, S7 and S11 have significantly higher viability than S5 (P<0.05) as they showed the viability of 62%, 59%, and 40%, respectively.

Resistance to intestinal juice was assessed on 0, 30, 60, 90, and 120 min (Figure 1B). As demonstrated, the S6 isolate had the lowest resistance to the simulated conditions of the intestine as the viability reached zero in 30 min. The viability of isolates 3 and 9 was zero following 60 min. in the present study, 3 and 9 were reported to be sensitive to the bile concentration of 0.3% and 0%, respectively. Viability of 4, 7, 12, and 14 reached zero in 90 min, all of which were sensitive to 0.3% bile except S14, which was found to be sensitive to 0.7% bile.

Finally, S5 and S11 isolates were able to tolerate intestinal simulated conditions with a 50% decrease in viability in 120 min. however, the mentioned isolates were not significantly different in terms of viability (P>0.05). Viability of 8 was zero after 120 min showing the lower resistance of this isolate, compared to S5 and S11.

Table 2 indicates the findings of the medium pH reduction test. Isolates 7 and 10 caused the highest and lowest pH decrease, respectively. Furthermore, isolates 3, 4, and 5 were not significantly different from 7 in this regard (P>0.05).

**Table 2.** Percentage of medium pH reduction by bacterial isolates derived from Naein traditional dairy product after 24 h of incubation at 37

| Bacterium code | Final pH | pH reduction percentage | Ranking |
|---------------|---------|-------------------------|---------|
| S1            | 5.67    | 8.8                     | 13      |
| S2            | 4.98    | 19.9                    | 11      |
| S3            | 3.63    | 41.6                    | 3       |
| S4            | 3.78    | 39.2                    | 5       |
| S5            | 3.67    | 40.9                    | 4       |
| S6            | 4.6     | 26                      | 8       |
| S7            | 2.67    | 57                      | 1       |
| S8            | 5.83    | 6.2                     | 14      |
Antimicrobial Activity

Findings of antimicrobial activity tests are demonstrated in Table 3. The highest antimicrobial effect was observed for S4 and S9 isolates (P<0.05). In other words, these isolates are the best choices for inhibiting *Salmonella typhimurium*. The bacterium *Pseudomonas aeruginosa* was significantly better inhibited by S4, S5, S12, and S14, compared to other isolates (P<0.05). Isolates S3, S6, and S8 significantly inhibited *Escherichia coli* (P<0.05). Moreover, *Staphylococcus aureus* was significantly inhibited by S7 and the yeast *Candida albicans* were inhibited by S3 and S14 (P<0.05).

### Table 3. Antimicrobial activity and ranking of isolates based on the diameter (mm) of the zone against pathogen bacteria

| Bacterium code | Final pH | pH reduction percentage | Ranking |
|----------------|----------|-------------------------|---------|
| S9             | 4.19     | 32.6bc                  | 7       |
| S10            | 5.94     | 4.5c                    | 15      |
| S11            | 3.39     | 45.4c                   | 2       |
| S12            | 5.17     | 16.8d                   | 12      |
| S13            | 3.91     | 37.1bc                  | 6       |
| S14            | 4.78     | 23.1bcd                 | 9       |
| S15            | 4.89     | 21.3bcd                 | 10      |

**P-value**

Values with different superscript letters in the column mean the significant difference between treatments (P<0.05)

Antibiotic Resistance

Table 4 demonstrates the results of antibiotic resistance for bacterial isolates from Naein traditional dairy product based on the diameter of the zone (mm). Most of the bacteria were sensitive or semi-sensitive to amoxicillin as S3, S4, and S5 were sensitive and isolates S6, S8, and S9 were semi-sensitive to amoxicillin. On the other hand, eight isolates were resistant to vancomycin and cephalaxin. Isolates S3, S4, S5, S7, S9, S11, S12, and S14 were resistant to vancomycin and cephalaxin.
were found to be resistant to vancomycin and S3, S5, S6, S7, S8, S11, S12, and S14 were resistant to cephalexin.

### Table 4. Antibiotic resistance of bacterial isolates from Naein traditional dairy product based on the diameter of the zone (mm)

| Isolate code | E15 Erythromycin | AMX 25 Amoxicillin | C30 Chloramphenicol | S10 Streptomycin | V30 Vancomycin | CN30 Cefalexin | FM300 Nitrofurantoin |
|--------------|-------------------|-------------------|---------------------|-----------------|----------------|----------------|---------------------|
| S3           | Resistant         | Sensitive         | Resistant           | Semi-sensitive  | Resistant      | Resistant      | Semi-sensitive      |
| S4           | Sensitive         | Sensitive         | Resistant           | Sensitive       | Resistant      | Sensitive      | Resistant           |
| S5           | Resistant         | Sensitive         | Resistant           | Resistant       | Resistant      | Resistant      | Resistant           |
| S6           | Resistant         | Semi-sensitive    | Semi-sensitive      | Resistant       | Resistant      | Resistant      | Resistant           |
| S7           | Resistant         | Resistant         | Resistant           | Resistant       | Resistant      | Resistant      | Resistant           |
| S8           | Resistant         | Semi-sensitive    | Resistant           | Semi-sensitive  | Resistant      | Resistant      | Sensitive           |
| S9           | Resistant         | Semi-sensitive    | Semi-sensitive      | Resistant       | Resistant      | Semi-sensitive | Resistant           |
| S11          | Sensitive         | Resistant         | Resistant           | Resistant       | Resistant      | Resistant      | -                   |
| S12          | Resistant         | Resistant         | Resistant           | Sensitive       | Resistant      | Resistant      | Sensitive           |
| S14          | -                 | Resistant         | Resistant           | Resistant       | Resistant      | Resistant      | Resistant           |

### Cholesterol Reduction

Results of cholesterol reduction by the bacterial isolates of Naein dairy product after 16 h of incubation at 37°C are shown in Table 5. Isolates S5 and S11 were significantly different from other isolates with reductions of 99% and 98%, respectively (P<0.05). The S8 and S9 with cholesterol reduction of 87% and 80% were not significantly different from S5 and S11 (P>0.05). Isolates S5, S11, and S8 had a suitable resistance to bile salts as could tolerate 1% of bile salts. The S9 and S14 could only tolerate 0.3% bile salt. Isolates S5 and S11 were reported to have suitable tolerance against acid conditions as could tolerate pH=2. Furthermore, isolate S12 was found to be able to tolerate acidic conditions up to pH=2.5. We observed that S5, S11, S8, and S9 has the highest cholesterol reduction levels. Isolate S12 was shown to impose the lowest impact on cholesterol reduction.

### Table 5. Cholesterol reduction percentage by bacterial isolates from Naein traditional dairy product after 16 h of incubation at 37°C

| Bacterium code | Cholesterol reduction percentage | Ranking |
|----------------|----------------------------------|---------|
| S5             | 99.14a                           | 1       |
| S8             | 87.32ab                          | 2       |
| S9             | 80.5a                            | 3       |
| S11            | 98.41a                           | 1       |
| S12            | 71.35b                           | 4       |
| S14            | 76.28b                           | 3       |
| P-value        | 0.012                            |         |
| Mean standard error | 5.571                   |         |

(Over 95% rank 1, over 85% rank 2, over 75% rank 3, and over 70% rank 4)

Values with different superscript letters in the column mean the significant difference between treatments (P<0.05)

Table 6 shows the findings of the bile salts hydrolase activity test. In this test, bile-resistant isolates (i.e., S5, S8, S9, S11, and S14), in addition to S12 as a negative control for controlling test accuracy were selected. The obtained results were reported as the measurement of the white zone produced by isolates in mm.

According to our findings, isolates S8, S5, and S11 had orderly the largest zones (P<0.05). Considering the cholesterol reduction test, these results could be
expected because the latter isolates had the highest percentages of cholesterol reduction. Activity of bile acids hydrolyzing enzyme was observed for the isolates, which could reduce blood cholesterol. Therefore, a correlation was suggested between these two features.

Table 6. Bile salts hydrolysis by the bacterial isolates of Naein traditional dairy product based on the diameter of zones

| Bacterium code | Zone diameter (mm) | Strong, weak, moderate | Ranking |
|----------------|--------------------|------------------------|---------|
| S5             | 3.5a               | Strong                 | 2       |
| S8             | 4c                 | Strong                 | 1       |
| S9             | 2.5a               | Moderate               | 4       |
| S11            | 3c                 | Strong                 | 3       |
| S12            | 1.5b               | Weak                   | 6       |
| S14            | 2.25b              | Moderate               | 5       |
| P-value        | 0.032              |                        |         |
| Mean standard error | 0.574            |                        |         |

Values with different superscript letters in the column mean the significant difference between treatments (P<0.05)

Molecular Identification of Bacterial Isolates with Probiotic Characteristics

Bacterial isolates S5, S8, S9, S11, and S14 had the potential for cholesterol reduction and bile salts hydrolysis. Moreover, S3 had higher antimicrobial properties and S7 was highly resistant to pH variations with strong antimicrobial impact. As a result, the aforementioned isolates were investigated using PCR for 16sr RNA for molecular identification. Results of sequencing were compared with the sequences in gene banks, including EzBioCloud (eztaxon) and NCBI. The final findings of sequencing for the evaluated isolates are summarized in Table 7. Consequently, Naein traditional dairy product had diverse genera of Lactobacillus, among which L. pentosus, L. crustorum, L. brevis, and L. fermentum were identified.

Table 7. Sequence BLAST of ribosomal 16s region of the DNAs of the isolates from Naein traditional dairy product according to the databases of NCBI and etaxon

| Bacterium code | Species in Taxon | Isolate | Similarity (%) | Species in NCBI | Isolate | Similarity (%) |
|----------------|------------------|---------|----------------|-----------------|---------|----------------|
| S3             | *Lactobacillus pentosus* | DSM 20314 (T) | 100 | *Lactobacillus Plantarum* | Strain H1, 16S ribosomal RNA gene | 100 |
| S5             | *Lactobacillus Crustorum* | LMG 23699 (T) | 99.93 | *Lactobacillus Crustorum* | Strain B481, 16S ribosomal RNA gene | 100 |
| S7             | *Lactobacillus fermentum* | CECT 562 (T) | 99.71 | *Lactobacillus fermentum* | Strain APBSMLB166, 16S ribosomal RNA gene | 99.71 |
| S8             | *Lactobacillus Pentosus* | DSM 20314 (T) | 99.93 | *Lactobacillus Plantarum* | Strain PS7319, 16S ribosomal RNA gene | 100 |
| S9             | *Lactobacillus fermentum* | CECT 562 (T) | 99.36 | *Lactobacillus fermentum* | Strain 10-18 16S ribosomal RNA gene | 99.36 |
| S11            | *Lactobacillus brevis* | ATCC 14869 (T) | 99.86 | *Lactobacillus brevis* | Strain NOS7311 16S ribosomal RNA gene | 100 |
| S14            | *Lactobacillus brevis* | ATCC 14869 (T) | 99.93 | *Lactobacillus brevis* | Strain SKB1021 16S ribosomal RNA gene | 100 |

Discussion

Evaluation of resistance to acidic conditions revealed that six out of 15 bacterial isolates had a suitable resistance to pH=2.5. Sharifi et al. (2017) investigated probiotic characteristics of the bacteria isolated from traditional yogurt in Yazd, Iran in pH=2.5-3. They reported lower resistance of Bifidobacteria, in comparison with Lactobacilli. In addition, they showed that Lactobacillus isolates were more resistant to acidic conditions than Streptococcus and Enterococcus. Overall, their findings were indicative of the diminished number of all isolates at pH=2.5 after 2 h.

Akbanda et al. (2013) demonstrated that at H=2.5, a decrease in the viability of bacteria was remarkable following 2 h, compared to 1 h (16). Consistent with our findings, they revealed that time was effective in the reduction of resistance and viability of isolates. The viability of isolates at pH=2 showed lower viability after...
120 min than 60 min. It could be attributed to the higher lysis rate of the bacterial cell wall by the acid in longer contact. Majidzadeh et al. (2011) investigated the impact of time and pH on the reduction of isolates activity. They reported that the isolates had a 55%-60% decrease in viability following 2 h of incubation at pH=2, which was in line with the findings of the current study (17).

Results of bile salts resistance assay showed that 60% of the isolates were sensitive to bile salts. Sharifi et al. (2017) tested 24 Lactobacillus isolates from traditional yogurt of Yazd, Iran using bovine bile extract 0.3% for 8 h. They reported 10, 2, and 12 isolates as resistant, highly resistant, and sensitive, respectively. Their results are consistent with the present investigation. Hajighassemi et al. (2016) recognized five resistant isolates following 8 h of incubation beside 0.3% bile salts and two resistant isolates utilizing 0.7% bile salts (8).

Isolates S8, S9, and S12, which were resistant to pH=2.5 but sensitive to pH=2, were destroyed after 120 min of exposure to gastric simulated conditions. As expected, isolates S7, S11, and S5, which were resistant to pH=2, were the most resistant bacteria to gastric simulated conditions after 2 h of incubation as they indicated 62%, 59%, and 40% viability. Gastric pepsin enzyme affects bacterial cell walls due to proteolytic activity and destroys the bacteria. A study on probiotic characteristics revealed that the viability of L. acidophilus and L. rhamnosus GG had a 60% reduction in gastric simulated conditions under treatment by the whole salt with pepsin enzyme for 120 min.

In intestinal simulated conditions, S6 had the lowest resistance as expected because the viability of this bacterium reached zero after 60 min in gastric simulated conditions and was not resistant to pH=5. Moreover, this bacterium could not tolerate 0.3% bile salts and was reported as sensitive. Another investigation on the viability of L. rhamnosus GG in intestinal simulated conditions demonstrated that the most reduction in viability occurred after 30 min of exposure to intestinal simulated conditions. This could be attributed to the sudden shock due to bacterial exposure to high pH and bile salts indicating the higher sensitivity of this bacterium to intestinal simulated conditions (19).

Evaluation of antimicrobial effects showed that isolates S9 and S4 were the best options for inhibiting S. typhimurium, S12 and S14 were the best for P. aeruginosa and isolates S3 and S8 were the most effective for E. coli. In addition, the best isolates for the inhibition of S. aureus were S7 and S9 and for C. albicans yeast were S3 and S14. The antimicrobial impact of some Lactobacillus isolates was correlated with pH decrease (20). However, the antimicrobial properties cannot be completed attributed to pH reduction. The secretion of bacteriocins as antibacterial compounds by Lactobacilli is believed to play role in this regard (21).

Numerous studies are still being conducted in this regard. Sharifi et al. (2017) reported a strong antimicrobial activity for L. rhamnosus and L. fermentum against the pathogenic bacteria S. typhimurium, S. aureus, and E. coli. An investigation was performed on the antimicrobial activity of Lactobacillus isolates from two samples of traditional yogurt. It was found that L. acidophilus with the mean diameter of 14.68 mm for the zone of inhibition against S. typhimurium and S. aureus along with L. plantarum with the mean diameter of 12.37 mm against P. aeruginosa had the highest antimicrobial activity. The mentioned findings were in line with the results of the present study.

A considerable aspect in terms of the safety of probiotics is antibiotic resistance. This is the potential risk of the transfer of antibiotic resistance genes among Lactobacilli. Following passing acidic conditions and bile in the digestive system, the transfer of antibiotic resistance genes located on plasmid to the flora of the digestive system and even intestinal epithelial cells is possible. As a result, the safety of using such bacteria as probiotics is reduced (22).

A study was performed on the resistance of Lactobacillus isolates from Mazandaran, Iran traditional cheese to the common antibiotics. It was observed that most of the isolates were resistant to streptomycin, vancomycin, and gentamycin, sensitive to amoxicillin, and semi-sensitive to nitrofurantoin (23), which is consistent with our results.

Tissay et al. (2014) in a clinical study, reported the influence of L. rhamnosus and L. bulgaricus on the diminish of blood cholesterol in rats. Furthermore, they stated that Pediococcus acidilactici could cause a 20% reduction in serum total cholesterol of rats (14). Shahat et al. (2016) evaluated the impact of probiotic isolates from a traditional fermented dairy product on health. These authors demonstrated that in laboratory conditions, isolates L. plantarum and L. brevis could reduce cholesterol (13).

Simultaneous with the enzymatic activity for further hydrolysis of bile salts by the isolates, cholesterol reduction and detoxification occurs in the digestive system, especially the liver. Comparison of the potential for bile salts indicates that the higher the ability of bacteria for hydrolysis of these salts, the better the reduction and detoxification by the digestive system and liver is performed (24).

Mirlohi et al. (2010) in a clinical study on laboratory mice, noted that L. plantarum was influential in the reduction of serum cholesterol. They considered having bile-hydrolyzing enzyme as one of the factors for cholesterol reduction by probiotic isolates, which caused an 8%-10% decrease in blood cholesterol (10).
Tissay et al. (2014) investigated bile salts derived from bovine bile in the diet of mice. They showed that L. acidophilus and L. fermentum led to the highest rate of serum cholesterol reduction, which was in line with the findings of the present study (isolate L. fermentum, S8). They attributed the decrease in serum cholesterol to the production of bile-hydrolyzing enzymes by Lactobacillus isolates (14).

**Conclusion**

According to the findings of the current study, koome is highly potential for isolating probiotic isolates and contains active probiotic isolates. Therefore, in the present investigation, isolates with probiotic characteristics were derived from this fermented dairy product of Naein. Isolates obtained from this product were demonstrated to have diverse potentials for healthy effects on the digestive system, such as having anti-pathogen activity, inducing acidic conditions, and hydrolyzing cholesterol.

**Conflict of Interest**

The authors declared no conflict of interest.
جذاب‌سازی بакتری‌های لاکتویواسی با قابلیت پروپیوتیکی از محصول لبنی سنتی نانین (کومه)

نینا شمشاد، لیلا رزبی نصیری ۱۳۹۹/۲۳۰۰، رضا مجیدزاده هروی

۱. دانشجوی دکتری، گروه علوم و صنایع غذایی، واحد نور، دانشگاه آزاد اسلامی، نوشهر، ایران
۲. استادیار گروه علوم و صنایع غذایی، واحد نور، دانشگاه آزاد اسلامی، نوشهر، ایران
۳. مدیر مرکز تحقیق و شفاهی‌سازی دانشگاه شهید باهنر کرمان، تهران، ایران
۴. استادیار گروه علوم دامی، دانشگاه گلستان، مشهد، ایران

چکیده
زمینه و هدف: کومه یکی از محصولات لبنی تجاری است که کمک به بهبود محصولات لبنی در روندهای اطراف نطفه می‌کند که به‌عنوان یکی از نوع کمپون‌ها توانایی هدف‌گذاری، جذاب‌سازی بکتری‌های لاکتویواسی را دارد. این بکتری‌ها می‌توانند رشد میکرو‌باتری‌ها را کنترل کنند و باعث نشهر می‌شوند.

مواد و روش: از محیط کشت MRS Agar برای جذاب‌سازی بکتری‌های لاکتویواسی استفاده شد. با استفاده از تحقیق‌های قبلی، در این تحقیق، بکتری‌های لاکتویواسی با استفاده از روش پیش‌بینیی با انسداد و استفاده از روش‌های مختلف، می‌توانند به‌عنوان بکتری‌های ویژه در محصولات لبنی استفاده شوند.

نتیجه‌گیری: این تحقیق نشان داد که کاربرد بکتری‌های لاکتویواسی در محصولات لبنی می‌تواند به‌عنوان یکی از روش‌های جدید جذاب‌سازی محصولات لبنی باشند.

کلیدواژه‌ها: پروپیوتیک، کومه، لاکتویواسی، محصول لبنی

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استادیار گروه علوم و صنایع غذایی، دانشگاه آزاد واحد نور، ایران
ایمیل: leila_roozbeh@yahoo.com

مقدمه
فرآورده‌های تخمیری مانند ماست، کنار، دوغ، سوارکار، کامبوچا و سایر محصولات لبنی که از قدمت بیش از سیصد سال در میان مردم مورد مصرف بوده است، امروزه به عنوان پروپیوتیک‌ی از راه‌پیمایی از کرده این مورد بررسی کرده است (۱). (علم پروپیوتیک درمانی) (Probiotic Therapy)، حاصل تکامل ایان روند در پروپیوتیک‌های شاید و مورد و تحقیقاتی روزگار (۲). (روزگار) از پیشگیری از بیماری‌ها در مورد سالمات معنوی و فیزیکی و بهبود توانایی و جلوگیری از بیماری‌ها در مصرف میان سیستم‌های بدن خاکستری (۴). (ماجرا) از پروپیوتیک‌های مناسب برای جذاب‌سازی محصولات لبنی می‌باشد.
گروهی از باکتری‌های گرم‌پس‌دار است که در فلور میکروبی دستگاه گوارش انسان وجود داشته و در بروز‌های تهیه مواد غذایی، کاربرد دارد و امروزه به‌عنوان حامی مختاری و با قابلیت تنظیم پایش‌های ایمنی، مورد توجه قرار گرفته‌اند. (5) باکتری‌های پروپیونیک طبق استانداردی می‌توانند به صورت ترش‌تاز که نه به‌یاسی در طول مدت زمان‌های متفاوت غذا زنده بمانند، بلکه باید در طول زمان مصرف‌رسان از اسید معدنی اتصال‌های قلیوی صورت پذیرند. به‌طور مثال، فعالیت گود (پلسترو) بر روی بدن دلیل گذشتی که ادعایی می‌شود دارای اثرات سلامتی بخش هستند، این هگام مصرف، حداکثر قانونی‌اند.

17 پروپیونیک زنده، در هر گرم وزنی بالند.

لاکتوسیلوس‌ها یا اولین بار بیش از چند جادوگری شده و امروزگر

پروپیونیک با فضای شریعتی همراه پیش داده‌ای از محصولات لینی

آگسازی، مانند کنار و دوگ بوه و بیش از 20 محصول قانونی با

بایوتی‌های باکتری‌های لاکتوسیلوس از افرادی است که به صیغه‌ای به‌طور مثالی از MRS Agar در این تحقیق، این مورد به صورت مثبت در رسته‌های ایرانی اثبات نشده، همچنین در مورد در این، از اسید معدنی اتصال‌های قلیوی صورت پذیرند. به‌طور مثال، فعالیت گود (پلسترو) بر روی بدن دلیل گذشتی که ادعایی می‌شود دارای اثرات سلامتی بخش هستند، این هگام مصرف، حداکثر قانونی‌اند.

*۱ Kwomeh
مجله میکروبیشناسی ایران

سعال ۱۶ شماره ۱ بهمن - اسفند ۱۳۹۹

رشد بزرگی گردید. ابتدا کشت فعال از باکتری‌های پروپیوتیکی، به کمک میکروگرایس در حضور نمکهای صترافی (یا اغلب آنزیم‌ها) با گلیسید ۴ میلی‌لتر در هر لوله فاکسون، تقسیم شد. بعد از اطمینان از خنک شدن میکروگرایس، ۲۰۰ میکرولیتر از کشت تازه و فعال باکتری پروپیوتیکی، داخل هر لوله فاکسون تهیه گردید و به خوبی مخلوط شد. پس از ۱۰ دقیقه، در دمای اتاق، قرار داده شدند تا با محیط هم‌گردد. سپس، محیط کشت طراحی شد و در هر ۲ ساعت نیمی از کشت باکتری مایع، کلریک وزنی در میکرومیکرگیسین بررسی می‌گردید. درصدهای تهیه شده، آرامی به باکتری گردیدن شد. این امر مشخص است که، با ظهور آنزیم، نیاز به رشد باکتری در محیط مایع، کلریک وزنی، افزایش می‌یابد.

 Shanasaieh, A. (2018). ارزیابی تاثیر ۱۰۰ درصد مایع در محیط کشت، با توجه به تغییرات در pH بررسی کامپوست فعال با کمک آزمایشگاه‌های اپتیکی تهیه گردید.

درصد مایع در محیط کشت، با توجه به تغییرات در pH بررسی کامپوست فعال با کمک آزمایشگاه‌های اپتیکی تهیه گردید.

درصد مایع در محیط کشت، با توجه به تغییرات در pH بررسی کامپوست فعال با کمک آزمایشگاه‌های اپتیکی تهیه گردید.
آزمون فعالیت هیدروولازی نیکه صفر اوی

بررسی‌های سلیمی در سوپروکتیکیل زیستی، در اطراف کلیه‌ها

در میانات کش‌شیتی نمایی نموده‌ای صفر اوی بردی ۱۰ میکروتریک‌نامه‌سازی را روی سطح پایه‌ای از آگر‌کش‌شیتی داده و در دما و زمان مناسب گرم‌خانه‌گیری MRS شدن. در صورت افکاری‌هایی در سوپروکتیکیل منفی، پروفسور و هم‌آفرین یا بر اساس موانع نیکه صفر اوی در اطراف کلیه‌ها و وضعیت است. از این رو تحقیق، در نظر گرفته شده‌است. در صورت عدم میکروتریک‌نامه، افتاده‌ها در سوپروکتیکیل بلافاصله آماده‌اند و ۷۰ میکروتریک‌نامه‌سازی بر روی سطح پایه‌ای از آگر گردیده. هم‌اکنون، به صورت تحقیقی در اطراف های سوپروکتیکیل نیکه صفر اوی، تست‌های دفاعی در آگر گردیده. با کردن مورد نظر (۱۰۰) نفر.

شناسایی جدول‌های انتخاب شده از آزمون‌های پروپتیکیل

همت جدول‌های که در آزمون‌های پروپتیکیل فوق برتری نسبی به سایر جدول‌های شرکت‌های را تعیین ترکیب DNA در ناحیه ۱۶۸ ریبووزیم شناسایی نمود. قطعه موزیک‌های به روش واسکنش زیست‌شناسی پیش‌تر با استفاده از دستگاه آزمایشگاه‌های رژیمی، مدل پالس تکپلر (PCR) تکپلر شد و پس از اجرای تکپلر (Corbett Life Science Pty. Ltd., Australia) ۵GAG AGT TTG ATC CTG GCT GAG

همت نور نظر داده (۱۰۰).

تخیل داده‌ها

دعاده‌ها حاصل از آزمایش‌های در این مطالعه، در قالب طرح

کامل تغییرهای با تکرار توسط نرم افزار SAS نسخه ۹۲ تجربی و
### جدول ۱

| ضریب بیازدادنی‌گی ۹۱ | ضریب بیازدادنی‌گی ۳۰ | کد باکتری |
|------------------------|------------------------|------------|
| -                      | -                      | S1         |
| -                      | -                      | S2         |
| -                      | -                      | S3         |
| مقاوم                    | مقاوم                    | S4         |
| حساس                    | حساس                    | S5         |
| مقاوم                    | مقاوم                    | S6         |
| حساس                    | حساس                    | S7         |
| مقاوم                    | مقاوم                    | S8         |
| حساس                    | حساس                    | S9         |
| مقاوم                    | مقاوم                    | S10        |
| حساس                    | حساس                    | S11        |
| مقاوم                    | مقاوم                    | S12        |
| حساس                    | حساس                    | S13        |
| مقاوم                    | مقاوم                    | S14        |
| حساس                    | حساس                    | S15        |

ازموم مقاومت به شیره معدن در زمان صفر، دقیقه، بررسی شد (شکل ۱). در این آزمون، منتخب‌های آزمون اسیدی و همین طور، منتخب‌های مرحله مقاومت S11 و S5 و همین طور منتخب‌های مرحله مقاومت S14 و S5 مرود آزمون قرار گرفتند. البته S14 سلسله S11، S8 و S5 مقدوم به صرف S11 و جدایی S3 و S4، S6 و S7 و S9، S6 و S4 مقدوم به دلیل مقاومت به pH=3.0 در این آزمون، مورد بررسی قرار گرفتند. نتایج به دست‌آمده در شکل ۱ ارائه شده است. همانطور که مشاهده می‌شود، جدایی‌های S4 و S6 کمترین مقاومت نسبت به شرایط شیب‌سازی شده معدن را داشتند. بطوریکه، بعد از ۴۰ دقیقه، درصد زندماني به صرف رسانده است. جدایی‌های S12 و S9، S8 و S7 مقدوم به حساس بودند، بعد از ۲۰ دقیقه مهم‌ترین مقاومت باید در خط روده (ب) طی مدت ۲ ساعت گرم‌خانه‌گذاری در دمای ۳۷ درجه سلزوس حروف کوچک مشابه رون ها اختلاف معنی‌داری با یا بدون نتایج به دست‌آمده (P<0.05) و شیره روده (ب) طی مدت ۲ ساعت گرم‌خانه‌گذاری در دمای ۳۷ درجه سلزوس حروف کوچک مشابه رون ها اختلاف معنی‌داری با یا بدون نتایج به دست‌آمده (P<0.05).
نینا شمشاد و همکاران | چداسازی اکتیواسیل‌های پروپیوتیکی از محصول لینی سنتی

زنده‌مانی قادر به حمل شرایط شیب سازی شده روده بوده ولی تفاوت معنی‌داری با یکدیگر از نظر درصد مقاومت نداشته‌اند (P > ۰/۰۵)، از جهتی S8 بعد از ۱۲۰ دقیقه، به صورت رسانه، نشان از مقاومت کمتر آن نسبت به S1 و S5 بود.

نتایج آزمون توانایی کاهش pH محیط در جدول ۲، قابل مشاهده می‌باشد. جدایی‌های S7 و S10 به ترتیب بیشترین و کمترین کاهش در محیط را داشتند. جدایی‌های S3، S5، S8، S4، S13، با لحاظ آماری تفاوت (P > ۰/۰۵) نشان دادند.

جدول ۲. درصد کاهش pH محیط توسط ایزوئیه‌های باکتریایی از محصول لینی سنتی نتیجه‌گیری‌های ۷۳ درجه سالمن دانشگاه تربیت مدرس

| رتبه‌بندی | درصد کاهش | تفاوت | کد باکتری | گروه‌بندی | نتیجه‌گیری |
|-----------|------------|-------|-----------|----------|-------------|
| ۱          | ٩/٠         |       | S1        |         |             |
| ٢          | ٧/١         |       | S2        |         |             |
| ۳          | ٦/٣         |       | S3        |         |             |
| ۴          | ٥/٨         |       | S4        |         |             |
| ۵          | ۴/۶         |       | S5        |         |             |
| ۶          | ٣/۶         |       | S6        |         |             |
| ۷          | ٣/۵         |       | S7        |         |             |
| ۸          | ۲/۷         |       | S8        |         |             |
| ۹          | ٢/۷         |       | S9        |         |             |
| ۱٠         | ٢/۷         |       | S10       |         |             |
| ۱١         | ۲/۷         |       | S11       |         |             |
| ۱٢         | ۲/۷         |       | S12       |         |             |
| ۱۳         | ۲/۷         |       | S13       |         |             |
| ۱۴         | ۲/۷         |       | S14       |         |             |
| ۱۵         | ۲/۷         |       | S15       |         |             |
| ۱۶         | ۲/۷         |       | P         |         | ارزش        |

حرکت کوچک مشاهده‌وی ستون‌ها اخلاقی معنی داری با هم ندارند (P > ۰/۰۵). (P > ۰/۰۵).

فعالیت‌های ضد میکروبی

نتایج آزمون توانایی کاهش pH محیط توسط ایزوئیه‌های باکتریایی از محصول لینی سنتی نتیجه‌گیری‌های ۷۳ درجه سالمن دانشگاه تربیت مدرس

(۲۰۲۱۰۵) بهترین نشان‌دهنده‌ای است. باکتری‌های دندان‌سوزی‌آمیز و تری‌نیزی از ترکیب جدایی‌های S11، S15، S7، S3، S14، S2، S12، S5، S4 و S10، به صورت رسانه و با نشان دهنده کاهش pH محیط، با لحاظ آماری تفاوت (P > ۰/۰۵) نشان دادند.

(۲۰۲۱۰۵)
و تحقیق برای تولید عقلانیت آنتی‌بیوتیک‌های جدید. (P<0.05)
جدول 4. مقاومت آنتیبوتیک‌های باکتریایی از محصول لبی سنتی نانی بر حسب قطر هاله (میلی‌متر)

| فیلم | نیتروفرانتوئین | سفید | سامونام | سترپومیسین | کلردمافنیولن | آمکسیلین | آریین
|------|-----------------|--------|---------|-------------|--------------|-----------|-----------------|
| FM300 | نیم‌حساس | حساس | مقاوم | نیم‌حساس | حساس | حساس | مثبت S3
| CN30 | مقاوم | حساس | مقاوم | حساس | حساس | حساس | مثبت S4
| V30 | نیم‌حساس | حساس | مقاوم | نیم‌حساس | حساس | حساس | مثبت S5
| S10 | نیم‌حساس | حساس | مقاوم | نیم‌حساس | حساس | حساس | مثبت S6
| C30 | نیم‌حساس | حساس | مقاوم | نیم‌حساس | حساس | حساس | مثبت S7
| AMX 25 | نیم‌حساس | حساس | مقاوم | نیم‌حساس | حساس | حساس | مثبت S8
| E15 | نیم‌حساس | حساس | مقاوم | نیم‌حساس | حساس | حساس | مثبت S9
| Erythromycin | نیم‌حساس | حساس | مقاوم | نیم‌حساس | حساس | حساس | مثبت S10

جدول 5. درصد کاهش کلسترول توسط ایزوله‌های باکتریایی از محصول لبی سنتی نانی بعد از 16 ساعت کرم‌خانه‌گازی در دما 37 درجه سلسیوس

| کد باکتری | درصد کاهش کلسترول | رتبه‌پذیری |
|-----------|-----------------|-----------|
| S5 | 99/14 | 1 |
| S8 | 88/33 | 2 |
| S9 | 87/50 | 3 |
| S11 | 81/41 | 1 |
| S12 | 87/35 | 4 |
| S14 | 87/28 | 3 |
| S15 | 87/20 | 5 |
| S16 | 87/12 | 6 |

میانگین کاهش استاندارد

(بلاگی 95٪ رتبه 1، بلای 85٪ رتبه 2، بلای 75٪ رتبه 3 و بلای 70٪ رتبه 4)

حرفوی‌های مشابه روستون‌های اختلاف معنی‌داری با هم ندارند (P>0.05).

نتایج آزمون فعالیت هیدروژن‌لیزری نمک‌های صفرائی در جدول 6، قابل مشاهده است. در این آزمون نیز، مانند روش کاشش کلسترول، جدایی‌های مقاوم به صفرائی (S14 و S11)، S9 و S8 و همچنین، S12 نیز، به عنوان کنترل منفی، جهت کنترل صحت آزمون، انتخاب گردیدند. نتایج حاصل شده طبق آنالیز گرفته گردید. این نتایج نشان می‌دهد که در بیشترین بخش‌های رنگ‌لیزری، از نوع کلسترول به‌طور کلی کاهش یافته است. یکی از اصلی‌ترین نتایج آزمون بود که کاهش در کلسترول به‌طور کلی مشاهده شد.
جلد 4، شماره 1، سال 15، شماره 1

بحث

بیدیو پاکت برخی از مولکول‌های تروکسولاریک، مولکول هایی با ساختاری مشابه به ژن تروکسولاریک و پلیمر لنزیک، ماهیگیر و اکثریت اورکاسیات، مولکول‌هایی هستند که با این ابزار می‌توانند میکروب‌ها را کنترل کنند. نوع مولکول‌های مذکور بسیار متفاوت می‌باشد و به گونه‌ای که می‌توانند باعث رشد میکروباگی‌ها یا کاهش آنها شوند. این اکثریت اورکاسیات می‌توانند باعث رشد میکروباگی‌ها یا کاهش آنها شوند. این اکثریت اورکاسیات می‌توانند باعث رشد میکروباگی‌ها یا کاهش آنها شوند.

جدول 6، نتایج بالا در تاثیر فعالیت اکسیدازی نسک‌های مختلف بر حالت فیزیولوژیکی اورکاسیات در میکرواروم

| کد باکتری | قطع هاله (mm) | ضعف هاله (mm) | قوی هاله (mm) |
|------------|---------------|----------------|---------------|
| S5         | 3             | 1              | 5             |
| S8         | 6             | 2              | 4             |
| S9         | 5             | 1              | 3             |
| S11        | 3             | 2              | 1             |
| S12        | 5             | 1              | 2             |
| S14        | 3             | 2              | 1             |

جدول 7، تاثیر تجویز 16S ribosomal DNA از اورکاسیات در میکرواروم

| کد باکتری | G. Plantarum | G. Crustorum | L. Pentosus | L. fermentum |
|------------|---------------|--------------|-------------|--------------|
| S5         | DSM 20314 (T) | L. Plantarum | DSM 20314 (T) | L. fermentum |
| S9         | L. Crustorum | L. Crustorum | L. fermentum | L. fermentum |
| S11        | DSM 20314 (T) | L. Plantarum | DSM 20314 (T) | L. fermentum |
| S14        | L. Pentosus | L. Crustorum | L. fermentum | L. fermentum |

دیل خاصیت ضد میکروبی بیشتر و S7 به دیل مقاومت زیاد به pH خاصیت ضد میکروبی قوی جهت شناسایی ملکولی با استفاده از واکنش زنجرهای پلی‌مرز 16S مورد بررسی قرار گرفتند. نتایج تعیین توالی (eztaxon) با توالی‌های موجود در بانکهای اطلاعاتی NCBI و EzBioCloud's مقایسه گردید. که نتایج توالی‌های نهایی می‌تواند در مورد باکتری‌های مورد بررسی از محصول لینی استنی نایین به عنوان واکنش‌های مشابهی باشد.
در ارتباط با کاهش زندگی بیشتری، pH 6/14 و شرایط باکتریایی نیز، بستگی به باکتری‌ها و شرایط زندگی آنها دارد. در شرایط سرطانی، pH 3/0 % باعث کاهش زندگی باکتری‌ها می‌شود و در شرایط نرم‌تر، pH 6/14 و شرایط باکتری‌ها می‌شود. در شرایط سرطانی، pH 3/0 % باعث کاهش زندگی باکتری‌ها می‌شود و در شرایط نرم‌تر، pH 6/14 و شرایط باکتری‌ها می‌شود. در شرایط سرطانی، pH 3/0 % باعث کاهش زندگی باکتری‌ها می‌شود و در شرایط نرم‌تر، pH 6/14 و شرایط باکتری‌ها می‌شود. در شرایط سرطانی، pH 3/0 % باعث کاهش زندگی باکتری‌ها می‌شود و در شرایط نرم‌تر، pH 6/14 و شرایط باکتری‌ها می‌شود. در شرایط سرطانی، pH 3/0 % باعث کاهش زندگی باکتری‌ها می‌شود و در شرایط نرم‌تر، pH 6/14 و شرایط باکتری‌ها می‌شود.
کنده صفاروی، نسبت داده شده که سبب کاهش 8 تا 10 درصد کلسترول سرم خون می‌شود (Tsai و همکاران، 2014). فعالیت این نکات باعثی برای جداسازی جایگاه رژیمی یا بوییونیک اکتیویتی را در کاهش کلسترول سرم خون می‌کند (1).

نتیجه‌گیری
در این مطالعه نشان داده شد که گیاهانی که در هر روز به آنها اضافه می‌شوند، جاده‌زدایی رژیمی بوییونیکی است و این روز رژیمی باکتریایی فعالی از نظر بوییونیکی دارد که در این تحقیق، جاده‌زدایی با خواص بوییونیکی از این محسول برخی حمایت ستی این جاده‌زدایی تردید. جاده‌زدایی بیشتر آدم از این محسول قابلیت‌های منفی را به ایجاد گیاهانی دستگاه جوارت فعالیت ضایعات، ایجاد شرایط اسیدی و همچنین قابلیت تجزیه کلسترول در ایجاد تندرستی که در اینجا مورد است. در اینجا معروف نشده است.

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توجه در منافع
در انجام این مطالعه، نیویسندگان هیچگونه تضاد منافعی نداشتند.

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