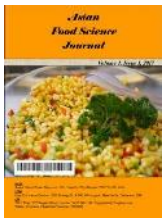

Asian Food Science Journal

3(3): 1-11, 2018; Article no.AFSJ.42292

Screening and Isolation of Functional Lactic Acid Bacterial Strains from Traditional Fermented Vegetables Juice (Jiangshui), Northwest China

Islam Rehmat[1], Xiangang Meng[1*], Xueping Li[2], Jing Wang[1] and Zhenzhen Mu[1]

[1]School of Chemical and Biological Engineering, Lanzhou Jiaotong University 730000, Gansu, China.
[2]Institute of Plant Protection, Academy of Agricultural Sciences, 730070, Lanzhou, Gansu, China.

Authors' contributions

This work was carried out in collaboration between all authors. Author IR designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors XM and JW managed the analysis of the study. Authors ZM and XL managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2018/42292
Editor(s):
(1) Dr. Iyekhoetin Matthew Omoruyi, Lecturer, Department of Biological Sciences, Benson Idahosa University, Benin City, Edo State, Nigeria.
Reviewers:
(1) A. Bamidele Tajudeen, Nigerian Institute of Medical Research, Nigeria.
(2) Libonatti, Carina, National University of Central Buenos Aires, Argentina.
(3) R. Prabha, Karnataka Veterinary, Animal and Fisheries Sciences University, India.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/25761

Original Research Article

Received 10th May 2018
Accepted 16th July 2018
Published 1st August 2018

ABSTRACT

Aims: Jiangshui (JS) is a traditionally fermented vegetable juice which is unique to northwest China as a seasonal beverage, especially during summer season. This study was designed to isolate functional lactic acid bacteria (LAB) from JS to investigate their probiotic properties as well as high cholesterol lowering capacity in human serum.
Design of Study: Lanzhou jiaotong university, Department of chemical and biological engineering, Laboratory of microbiology and food biotechnology, Lanzhou city, Gansu, between March and January 2018.
Methodology: The strains were initially screened by MRS culture medium and high cholesterol culture medium. Then, strains were confirmed by microbiological, biochemical analysis as well as 16S recombinant DNA sequencing.
Results: After investigating different parameters of isolated LAB strains such as bile tolerance,

**Corresponding author: Email: mengxg1@163.com;*

acid resistance and antimicrobial drug sensitivity, each strain have the capacity of cholesterol degradation. The results showed that the four strains with cholesterol reduction rate (75%) were mostly identified as *Lactococcus lactis ssp. lactis* (M2- JQ953697.1), two of them were *Brevibacterium casei* (M5 and H4- JF951998.2) and one was *Lactococcus raffinolactis* (Q7- KC951926.1.

Conclusion: In conclusion, JS is one of the best source for its nutritional values and its potential for cholesterol lowering capacity in human serum, especially M2 and Q7 strains have strong ability to survive at lower acidic conditions of gastric juices and tolerate the bile salts.

Keywords: Jiangshui; LAB; traditional fermented juice; cholesterol-lowering capacity; probiotic.

1. INTRODUCTION

Jiangshui (JS) is a naturally fermented juice obtained from vegetables such as Chinese cabbage, celery and wild herbs which is edible food for locals in north-western China [1,2]. They have a long history of consumption and production of JS for more than two thousand years. Initially JS was homemade with high risk of contamination. But recently, some of the progress in industry and production standard of traditional fermented vegetables have got attention [3]. The climatic characteristics of the area is temperate and semi-arid which is in favor for JS production. As compared to other fermented vegetable products, JS is appreciated by locals as a fermented juice, which is used as a seasonal drink. It is cool sour juice available as nectar which has attractive fragrance and is mostly utilized by people in the hot summer as a refreshing and flavored drink to quench their thirst [4].

Since last 30 years, a high number of people eating fatty and cholesterol enriched food that leads to atherosclerosis and hypertension [5]. Under certain circumstances, cholesterol in a body has a very important physiological functions but due to modern life style and diet changes, excessive intake of cholesterol has caused certain harmful effects to the human body. High serum cholesterol is an important factor to provoke cardiovascular diseases [6], which has become the main cause of death in China [7] as well as the global threat to human health. Some research statistics shows that, cholesterol level in human serum has decreased to 1% which has alternatively decreased cardiovascular problems to 2-3% [8]. According to a recent finding, Cholesterol level can be reduced by means of certain dietary and pharmacological ways; first, practice low-fat diets for long-term conditions. Although it is quite effective approach for prevention of atherosclerosis but it seems quite challenging and difficult to maintain [9]. Secondly, many cholesterol-lowering medications such as statin even though it helps to lower the cholesterol level in blood. Unfortunately, long-term use of statin has been hindered by certain adverse effects with serious consequences such as skeletal muscle toxicity [10] and also elevate transaminases level which can cause serious damage to liver. Therefore, finding a safe and reliable method to reduce serum cholesterol in food and human body has become a problem for many researchers [11].

JS has certain advantages over other Chinese fermented foods as it has low calories and is a major source of carbohydrate and vitamins [12]. JS juice is a rich source of lactobacilli like Korean kimchi and many strains have been confirmed clinically effective and possess several health benefits including improved gastrointestinal functions by resisting harmful bacteria [13], provoke immunity and curing hypertension [14, 15]. Previous studies have shown that some strains of LAB such as *Lactobacillus plantarum* can reduce cholesterol in human [16], as well as *lactobacillus acidophilus* was isolated from intestinal tract of pigs which can reduce cholesterol level in serum [17]. They isolated *lactobacillus* from fermented milk which have cholesterol lowering properties in vitro [18]. Thus it is proved that screening of probiotic LAB has the ability to degrade cholesterol and easy to apply for food processing, which will have a great effect on the development of food industry. In addition, LAB present in fermented food can control serum cholesterol concentration which can reduce the cardiovascular diseases and improve health. It is rarely found in previous studies that JS can degrade cholesterol level in human serum. However, as compared to other fermented vegetables, the research work on JS is still in development, especially the production process has still a comparatively low profile at industrial level.

To our knowledge, however, little information is available regarding cholesterol lowering

characteristics of LAB isolates from JS in Gansu, China. The objective of current study was to screen LAB strains isolated from traditional fermented vegetables juice called JS, and to evaluate these LAB isolates for probiotic characteristics and cholesterol lowering potentials in human serum.

2. MATERIALS AND METHODS

2.1 Equipment and Reagents

pHS-3C digital acidity meter (Aolilong instrument Co., Hangzhou, China); centrifuge 5418R (Eppendorf company, NY, USA); Mycycler PCR (Bio-Rad life medicine Co. California, USA); Gel electrophoresis HE-120 and Gel imager 250 (Tanon sci & tech Co., Shanghai, China); Nano Drop 2000 UV spectrophotometer (Thermo Fisher Co. Wilmington, USA); Sorvall legend RT-centrifuge (Kendro lab products Co. USA); Bovine bile salt and cholestrin (Zhongqin chemical reagent Co., Shanghai, China); antimicrobial drugs (Microbial reagent Co., Hangzhou, China); CTAB, EDTA, Tris-saturated phenol (Sigma Co. US); agarose (Sangon Biotech Co., Shanghai, China).

2.2 Sample Collection

The JS 30 samples were collected from five different regions of northwest China. The sample collection sites were: 1) Kongjia market, Anning district (N 36°05′, E 107°.88′), Lanzhou city, Gansu 2) Lintan (N 34°69′, E 103°35′), Gannan city, Gansu, 3) Binhe town, Shapotou district (N 37°45′, E 105°03′), Zhongwei city, Nigxia, 4) Wujia dun village, Qingyang city (N 36°03′ E 107°88′), Gansu 5) Qin'an village, Tianshui city (N 34°89′ E 105°69′), Gansu. All samples were collected in sterile plastic bags and kept in small containers (4 °C) in car. After transporting to the laboratory, the samples were stored at lower temperature.

2.3 Primary Screening and Isolation of LAB Strains

For isolation and enumeration of LAB, the JS samples (2 g) were thawed at room temperature and serially diluted to the appropriate concentration (10^{-4}-10^{-6}) under aseptic conditions. After that, the diluted samples were initially inoculated onto de Man, Rogosa and sharp (MRS) agar and liquid medium, which were used as universal culture medium for lactic acid bacteria, cultivated microaerophilic and incubated at 37°C for 48 h. After incubation, the colony was subculture on calcium carbonate medium (2%), to isolate by identifying the transparent ring appearance on the surface due to free bile acids. The ability to degrade cholesterol is proportional to the size of transparent ring [19]. After incubation at 37°C for 24 h, the single colonies that dissolved calcium were picked and streaked across the agar plate surface for purification. All the isolates were subjected to morphological and microscopic examination such as Gram staining and catalase test, and strains showing Gram positive and catalase negative were suspected to be LAB strains. Four strains were confirmed with 16S recombinant DNA gene analysis.

2.4 Cholesterol Standard Curve

MRS broth culture medium was supplemented with 0.2% (w/v) bovine bile salts, 0.2% sodium thioglycolate and 0.01 g/mL of cholesterol solution. After filtration, 2% (v/v) solution was added to the culture medium. Finally, cholesterol standard curve was determined.

2.5 *In-vitro* Screening of Cholesterol Reducing Strains

The screened strains were inoculated twice on a high cholesterol medium and incubated at 37°C for 48 h, After centrifugation (centrifuge 5418R). The OD_{600} (optical density of samples at 600 nm) values were measured by o-phthaldehyde method at 0, 12, 24, 72, h interval [20]. The cholesterol concentration and degradation rate were calculated according to the standard curve. The strains with relatively high degradation rate were screened out for further experiment.

2.6 Determination and Mapping of LAB Growth Curve

The broth of each strain with high cholesterol concentration was spread on *MRS* medium with 10% inoculum and cultured for 24 h for three generations. From start, the broth was plated every 4 h and each strain was subjected to 5 replicates and incubated at 37°C for 24 h. The concentration and mean value was monitored by measuring with the growth curve.

2.7 Acid-resistant Properties

To screen the acid tolerance of LAB strains, the broth of each strain was inoculated into MRS medium with high cholesterol and pH was

adjusted to 1.5, 2.5, 3.5 and 4.5 with 10% inoculum. The culture medium was incubated at 37°C for 24 h and the OD_{600} values were measured with Nano Drop 2000 UV spectrophotometry (Thermo Fisher Co. Wilmington, USA) after every two-hour interval.

2.8 Resistance to Bile Salts

Bile tolerance of screened strains was measured as described by [21]. The fermented broth of each strain with high cholesterol concentration was inoculated into MRS medium containing 2%, 4%, 6%, 8% with 10% sodium chloride and 10% inoculum which was incubated at 37°C for 24 h. Secondly, to evaluate bile salt tolerance, each fermented bacterial broth was inoculated into MRS medium with 0.1%, 0.2%, and 0.3% bile content and 10% inoculum. The culture media was incubated at 37°C for 24 h and the OD value was measured under 600 nm after every two h interval with spectrophotometer. Growth curves were plotted by the increase of OD_{600} against incubation time. All the experiments were measured twice.

2.9 Antimicrobial Drug Sensitivity

High cholesterol bacterial culture medium was mixed with MRS at 45°C and poured into sterilized culture dish. The five antimicrobial sensitive drugs (Hangzhou microbial reagent Co., Guangdong province, China) were placed on the plates including ampicillin, tetracycline, gentamicin, erythromycin and penicillin and incubated at 37°C for 24 h. Finally, the growth of lactobacilli was observed on basis of inhibition zone. The diameter of inhibition zone was measured with a Vernier caliper and the antibiotic resistance was determined.

2.10 Antibacterial Properties

The *Escherichia coli* and *Staphylococcus aureus* were activated and cultured. Each 1 mL was coated on the MRS medium plate. The sterilized Oxford Cup was placed on the culture medium plate with 50 µl fermentation broth, 50 µl the supernatant of cholesterol lowering strains and 50 µl sterilized fermented broth was poured into Oxford cup for 24 h at 37°C. Finally, the size of the loop, inhibition zone was analyzed and recorded.

2.11 Physiological and Biochemical Characteristics

The strains were subjected to Gram staining, catalase reaction test, starch hydrolysis test, gelatin liquefaction test, litmus milk test, sugar fermentation test, hydrogen sulfide production test, indole production test and acetyl methyl carbinol (V-P) test and the experimental strains were observed and recorded as shown in Table 1.

2.12 Molecular Identification of LAB Strains by 16S rDNA

The DNA of each bacterial strain was extracted with bacterial DNA extraction kit (Huada gene Co. Beijing, China). The purity and concentration of DNA were analyzed using agarose gel electrophoresis (Tanon sci & tech Co., Shanghai, China) and Nanodrop 2000 UV spectrophotometry respectively. The universal primers were used for amplification of the bacterial 16S rDNA 27F 5-AGAGTTTGATCCTGGCTCAG-3´ AND 1492R 5-GGTTACCTTGTTACGACTT-3´, synthesized by (Huada gene research center, Beijing, China).

The bacterial PCR amplification reaction was performed in a total volume of 25 µl containing 2.5 µl of 10X PCR reaction buffer solution; 10 mmol/L Mg 2.5 µl, 25 mmol/L DNTP's 0.5 µl, 20 µmol/L PCR primer each 0.5 µl, 5 U/µl Taq DNA polymerase 0.25 µl, 25 ng/ µl template DNA 2.0 µl and sterile steamed diluted water to 25µl. The PCR reaction conditions were as follows: amplification was performed as initial pre-denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 60 s and elongation at 72°C for 30 s, 40 cycles of amplification were repeated. Final, elongation was performed at 72°C for 10 min and preserved PCR product was stored at 4°C till electrophoresis. The sequencing of the amplified products was directly done by Beijing Huada gene research center.

2.13 Statistical Analysis

The data was analyzed by using Excel data sheets and IBM SPSS statistics software 19.0 version. Simpsons diversity index was used which quantifies the concentration of culturing microorganisms in JS. All sample analysis for various morphological and biochemical was performed in triplicate and reported as mean value. Statistical difference was defined $P>0.05$.

2.14 Construction of Phylogenetic Tree

The phylogenetic tree was constructed by N-J adjacency method using ClustalX 1.83 and

MEGA 5.3 software and the sequences were compared against Genbank data and found 99% similarity.

3. Result and Discussion

3.1 Screening of LAB

The colony morphology and Gram staining results shows that isolated strains were Gram positive, moist, white color, round, large and clear colonies. The cholesterol standard curve was plotted with high cholesterol concentration (g/ml) as horizontal coordinate with absorbance of A_{550} nm. The regression equation was:

$$Y= 3.86 \times 10^{-3}X + 4.28 \times 10^{-4}.$$

R2 = 0.995 (curve estimation was 0.995, linear estimate was 0.998). (1)

3.2 Cholesterol Degradation Rate

Selected strains have a certain ability to reduce cholesterol level and the overall reduction rate was increased with the incubation time as shown in Fig. 1. However, the cholesterol degradation rate of different strains was quite dissimilar. Such as L14 have 25% low degradation rate for 72 h while the strain M2 reached more than 90% with three times degradation ability. In this study, four strains with final degradation rate exceeding 75% were identified and the final degradation rate of M2 was 92.28%, which was higher than the previous studies.

3.3 Growth Curve of High Cholesterol Degrading Strains

The growth curve of the strains was slightly different from each other but overall growth rate showed a similar pattern as shown in Fig. 2. The average growth period was in a range of 0-20 h, the stationary phase between 20-32 h and enters in decline phase after 32 h. Results provided were presented as the mean value and concentration with the growth curve depending on the parameters characterized and test applications.

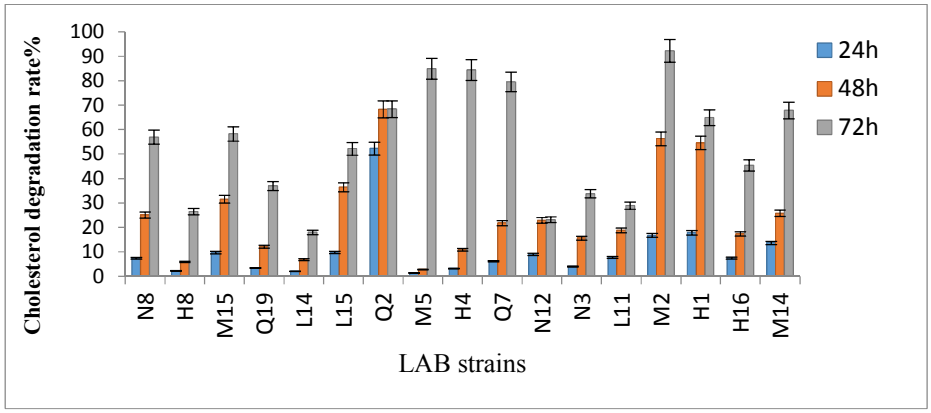

Fig. 1. Cholesterol degrading rate of the isolated strains

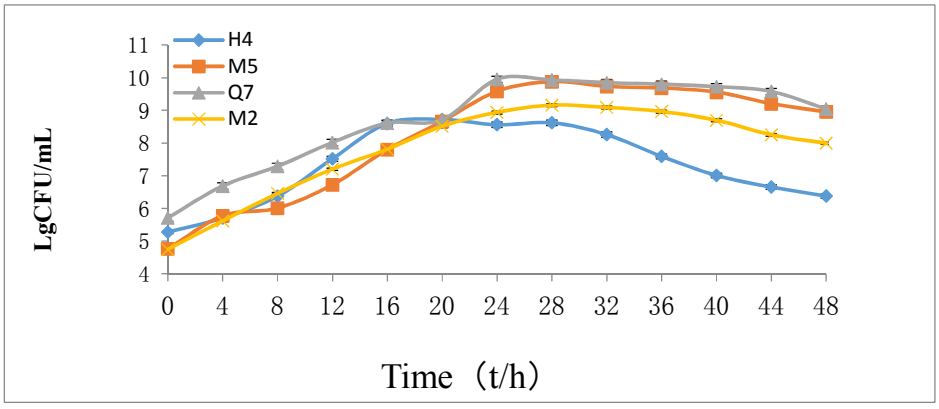

Fig. 2. Growth curve of cholesterol degrading strains

3.4 Salt Tolerance and Acid Resistance

The salt tolerance of each strain was different. Specifically, M2 and Q7 was growing normally at 2% and 4% *NaCl* concentrations. While the growth was inhibited at higher salt concentrations such as M5 and H4 which were salt-intolerant strains shown in Fig. 3. All strains showed a certain degree of acid resistance but grow better at high pH. However, different strains have different tolerance ability against acids and the Q7 strain was restrained at pH 1.5. Although other strains have certain resistance ability in acidic environment at pH 1.5 and 2.5 and the growth was inhibited to adopt for a longer period.

3.5 Bile Salt Tolerance Properties of Strains

It is clear that M2 and Q7 strains have good ability to salt tolerance. They can survive at different concentration but favorable growth was found at lower bile salt concentration. On the other hand, H4 and M5 strains can tolerate bile salt but the lag period was too long. Hence, the significance of practical application is not high.

3.6 Antimicrobial Drugs Resistance to High Cholesterol Degrading Strains

All experimental strains belong to multi drug resistant. The results showed relatively different responses to antibiotics sensitivity and even the same strains have different sensitivity to various antibiotics as mentioned In Fig. 4. There was no inhibitory zone observed around the drugs-sensitive sheet of Cefazolin, tetracycline, penicillin, ampicillin and erythromycin of Q7 and M2 strains (The diameter of the drug-sensitive sheet was 6 mm). It was finally determined that three bacterial strains have resistance to five kinds of antibiotics, which belongs to multiple drug resistant strains except H4 and M5 which was sensitive to cefazolin and resistant to other four kinds of antibiotics. The inhibition zone of antimicrobial drug resistance was dissimilar and showed resistance to all four kinds of strains, therefore it belongs to multiple resistant strains.

3.7 Antibacterial Properties

The bacterial fermentation broth, supernatant and sterilized fermentation broth were initially centrifuged 10000 rpm for one minute and sterilized by using sterile filter. Each strain of fermentation broth, supernatant and sterized fermentation broth was found different from those of *Escherichia coli* and *Staphylococcus aureus*. The strongest antibacterial property of fermentation broth was found of M2 bacteria as shown in fig. 5. In addition, the fermented supernatant of experimental strains had certain inhibitory effect on *Escherichia coli* and *Staphylococcus aureus*, indicating that the bacteria produced some antimicrobial substances during the growth phase.

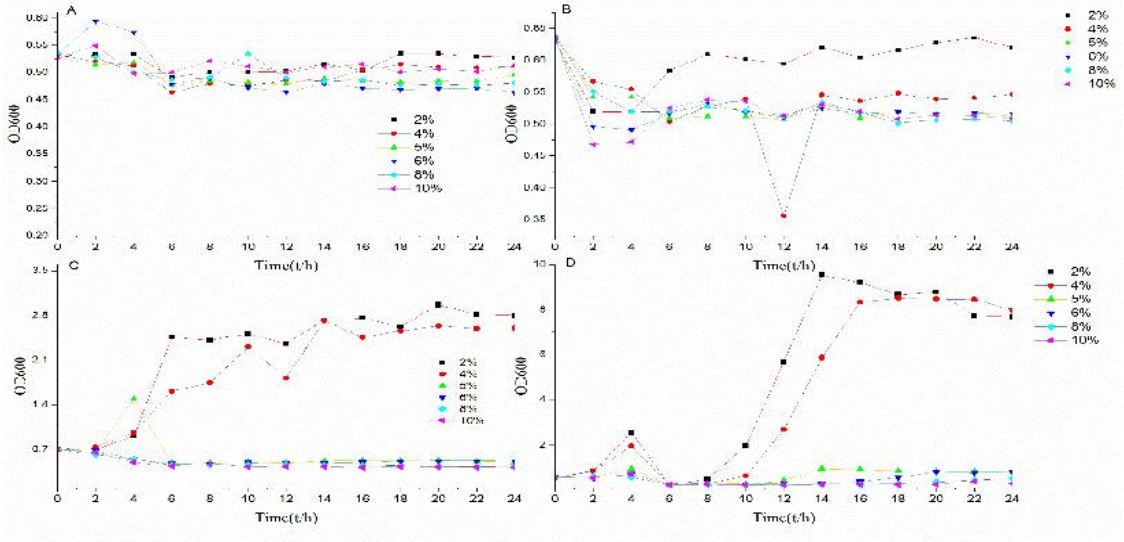

Fig. 3. The growth condition of strains with different bile salt conditions. a) M4 strain, b) H4 strain, c) Q7 strain, and d) M2 strain

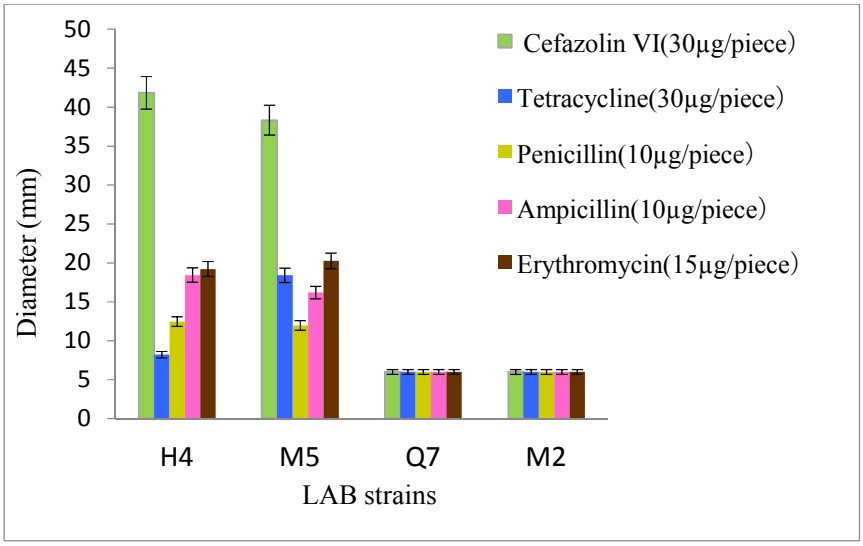

Fig. 4. The sensitivity of different antimicrobial drugs to LAB strains by measuring the zone of inhibition

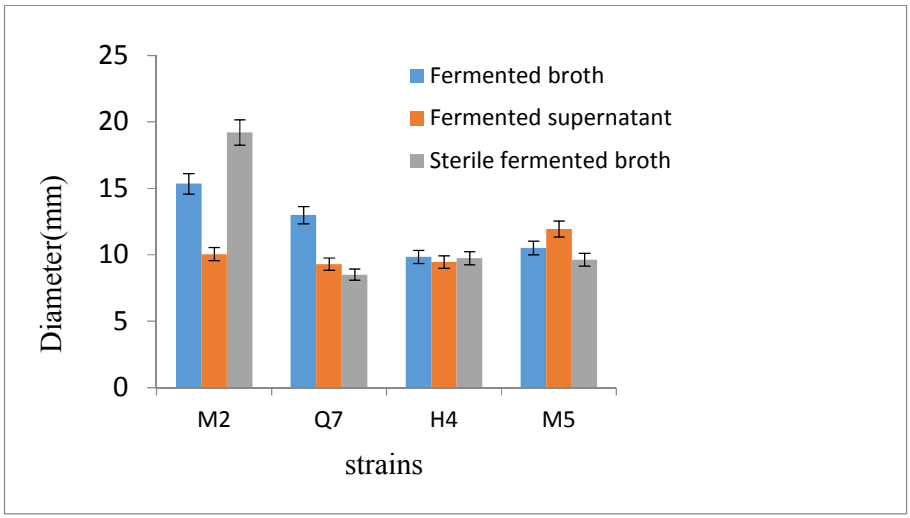

Fig. 5. Antibacterial properties of LAB strains in fermented broth

3.8 Physiological and Biochemical Properties

As shown in Table 1, physiological and biochemical characteristics of the different strains were compared with "Bergey's manual of bacterial identification" and "common bacterial identification manual". Different biochemical tests were carried out and finally the results showed that M2 was identified as *Lactococcus lactis*, *M4 and* M5 was *Brevibacterium casei* and Q7 *Lactococcus raffinolactis*.

3.9 Molecular Identification of Strains by 16S rDNA Sequencing

After DNA extraction and 16S rDNA amplification, the results were approving that the size of genomic DNA was about 23 kb and no light dispersion was observed in running the sample. The whole bands were intact with no smearing. The OD260 and OD280 were measured through UV spectrophotometry and calculated values were in the range of 1.8 to 2.0. The values within this range were selected for analysis and the concentration of DNA was between 40-80 ng/L. It

Table 1. The pysiological and biochemical charaterization of experimental LAB strains

Strains	Contact enzyme	Starch hydrolysis	Gelatin liquefaction	Litmus milk	V-P test	Sugar fermentation					Indole produce	H_2S produce
						Glucose	Galactose	Sucrose	Maltose	Lactose		
M2	-	-	-	SN*	-	+	-	-	+	+	-	-
M5	+	+	+	SD**	+	+	-	-	-	-	-	-
H4	+	+	+	SD	+	+	-	-	-	-	-	-
Q7	-	-	-	SD	+	+	-	-	-	+	-	-

***: SN means acid coagulation; ** SD means acid peptonized.*

Table 2. Molecular identification results and homology of LAB strains with Genbank data

Strains	Physiological and biochemical identification	Molecular identification	Similarity	Log in ID
M2	*Lactococcus*	*L. lactis ssp. lactis*	100	JQ953697.1
M5	*B.casei*	*B.casei*	100	JF951998.2
H4	*B.casei*	*B.casei*	99	JF951998.2
Q7	*L.raffino lactis*	*L.raffino lactis*	100	KC951926.1

is concluded that the proposed combining electrophoresis of DNA purity is high, RNA and protein contents were in a low amount with no contamination. Overall, the proposed DNA has good integrity and high purity which meets the requirement of research. The prokaryotic universal primer 16S rDNA sequences were used for PCR amplification reaction with the target fragments between 1000 to 2000 base pairs, with an average size of 1400 base pair. Banding was clear and bright with no dispersion zone and has good integrity.

According to the results of physiological and biochemical identification, four strains were compared with "Bergey's manual of bacterial classification" and homology was confirmed by 16S rDNA sequencing as shown in Table 2. M2 was *Lactococcus lactis* ssp. *lactis,* M4 and M5 were *B. casei* and Q7 was *L. raffinolactis.* This is the first case reported on the serum cholesterol degrading strains in JS and most of the isolated strains sequences were identical to the sequences obtained through Genbank and all isolated microorganisms belong to lactobacilli. First, random selection was carried out by proportionally isolating colonies on MRS culture medium. Next physiological and biochemical characteristics of experimental strains were analyzed as well as 16S rDNA sequencing was performed for screening of lactobacilli strains.

JS is the best probiotic lactobacilli source with having multiple functions, which can improve gastrointestinal health by enhancing the quality of vegetables and ensuring safety [22; 23]. There is preliminary evidence that the use of probiotic lactobacilli and its metabolic by-products potentially confer benefits to the heart including reduction of serum cholesterol [24]. Kimchi, a traditional Korean fermented food having probiotic properties and fermented by various kinds of lactobacilli strains, which have same probiotic properties comparing to JS. *L.citreum* is the best starter for quick fermentation in production of kimchi [25], health functionality of kimchi like anti-cancer, serum-cholesterol reduction and immune boosting [26].

As mentioned above, the growth curves of four strains were significantly similar to the typical growth curves that show the lag phase of microorganisms. The reason was the amount of inoculation in this study was high (10%), both culturing and activation was carried out three times. Nutrient broth and the experimental medium were same, therefore the time required for microorganisms to adapt the new environment was short and no significant delay was observed in specific time period. On the other hand, pH of the normal human body gastric juice fluctuates from 1.5 to 2.5 and most of the microorganisms are difficult to survive at lower acidic environment [27]. Some strains of lactobacilli can be take into account for survival in such conditions of gastric juices. Therefore, current study focus on the acid resistance properties of the strains, Q7 strain has strong resistance and can easily adapt the acidic environment of gastric juices. In addition, *LAB* and other probiotics can be colonized to human intestinal tract which in turn have certain tolerance ability to bile salts. This tolerance directly determines the survival time of bacteria in the intestinal tract. In this study, strain M2 and

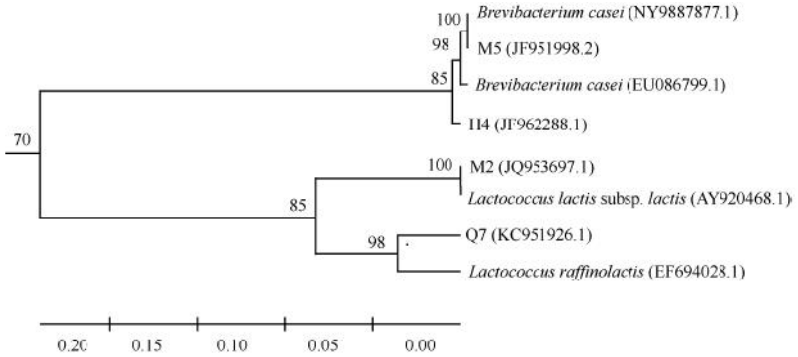

Fig. 6. Phylogenetic tree of the isolated strains designed by N-J method. M5, H4, M2, Q7 stand for the experimental strains. The serial numbers in the brackets are registered from Genbank. The length of branches indicates evolutionary distance. The data at branch, e.g. 70,85 etc, are confidence coefficient. The line segment below act as genetic distance scale and the numbers on the scale show genetic distance

Q7 have a strong ability to tolerate the bile salts of human intestine with a concentration (0.03% to 0.30%). Finally considering that Q7 strain have great potential for development but other strains can still grow by passing through the adaptation period which can also be used as source of industrial beneficial strains.

In recent years, scientists have found that some probiotics have been resistant to antibiotics, but there is still different understanding on this issue and most researcher are worried about it because once the probiotics loaded with drug resistant gene which may not only transfer the gene but they can also propagate a large number of harmful bacteria with no available treatment. Furthermore, it is also possible that, after entry of probiotics to intestinal tracts it can play a beneficial role with unlimited growth and production and turn from a guest into host cycle [28]. In this study several strains of bacteria have different levels of drug resistance. Generally, it is believed that if the choice of antibiotics in the intestinal environment is not high, there are less chances about the transmission of the antibiotics resistant genes of probiotics [29]. In previous studies, less functional probiotic strains have been isolated from JS [2]. In this study, the strains degrading cholesterol were studied and obtained significant results. In addition, it was found that LAB strains isolated form JS have the ability for cholesterol reduction in human serum. These results have confirmed the significant effects and JS potential for further development.

4. CONCLUSION

In conclusion, the identification results of bacterial strains showed that JS is indeed a good fermentative vegetable juice for reduction of cholesterol in human serum and also improve gastrointestinal functions. The bacterial isolates revealed that JS has attracted considerable interest due to its remarkable nutritional values and its potential functional probiotic properties helping in cholesterol reduction. It is also noteworthy that the cholesterol lowering activity of each strain is different.

ACKNOWLEDGEMENT

We would like to thanks Ms. LXP for guidance and technical assistance regarding analysis of JS. Besides, an honorable mention goes to our friends and families for the understanding and support to accomplish this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhang Y, Wang YL, Chen XQ, Zhao P. Isolation and initiative identification of microorganism from traditional fermentative food—Jiangshui. Food Sci. 2007;28(01):219-222. Chinese.
2. Li XP, Li JH, Meng XG, Zheng N, Xie JJ. Isolation and identification of microorganisms from Jiangshui, a traditional Chinese fermented vegetable product. Food Sci. 2014;35(23):204-209. Chinese.
3. Hou Z, Yang J, Jia H, Lu X, Wang L. Research overview and prospect of traditional fermented food—serofluid dish. China Condiment. 2015;40(02):132-136. Chinese.
4. Yoon KY, Woodams EE, Hang YD. Production of probiotic cabbage juice by lactic acid bacteria. Bioresour. Technol. 2006;97(12):1427-1430.
5. Lye HS, Rahmat-Ali GR, Liong MT. Mechanisms of cholesterol removal by lactobacilli under conditions that mimic the human gastrointestinal tract. Int. Dairy J. 2010;20(3):169-175.
6. Manson JE, Tosteson H, Ridker PM, Satterfield S, Hebert P, O'Connor GT, et al. The primary prevention of myocardial infarction. N. Engl. J. Med. 1992; 326(21):1406-1416.
7. He J, Gu D, Wu X, Reynolds K, Duan X, Yao C, Wang J, Chen CS, Chen J, Wildman RP, Klag MJ, Whelton PK. Major causes of death among men and women in China. N. Engl. J. Med. 2005;353(11): 1124–1134.
8. Anderson JW, Gilliland SE. Effect of fermented milk (yogurt) containing Lactobacillus acidophilus L1 on serum cholesterol in hyper cholesterolemic humans. J. Am. Coll. Nutr. 1999;18(1):43-50.
9. Anandharaj M, Sivasankari B, Parveen Rani R. Effects of probiotics, prebiotics, and synbiotics on hypercholesterolemia: A review. Chin. J. Biol.; 2014.
10. Beltowski J, Wojcicka G, Jamroz-Wisniewska A. Adverse effects of statins-mechanisms and consequences. Curr. Drug Saf. 2009;4(3):209-28.

11. Ballantyne CM, Blazing MA, King TR, Brady WE, Palmisano J. Efficacy and safety of ezetimibe co-administered with simvastatin compared with atorvastatin in adults with hypercholesterolemia. Am. J. Cardiol. 2004;93(12):1487-1494.
12. Liu R, Dang S, Yan H, Wang D, Zhao Y, Li Q, et al. Association between dietary protein intake and the risk of hypertension: a cross-sectional study from rural western China. Hypertens. Res. 2013;36(11):972-979.
13. Zhang Z, Chen Z, Gong X. Microflora analysis in the fermentation of Jiangshui [J]. Chin. Brew. 2009;10:012. Chinese.
14. Liu SN, Han Y, Zhou ZJ. Lactic acid bacteria in traditional fermented Chinese foods. Food Res. Int. 2011;44(3): 643-51.
15. Wang Y, Xu N, Xi A, Ahmed Z, Zhang B, Bai X. Effects of Lactobacillus plantarum MA2 isolated from Tibet kefir on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. Appl. Microbial. Biotechnol. 2009;84(2):341-347.
16. Jeun J, Kim S, Cho SY, Jun HJ, Park HJ, Seo JG, et al. Hypocholesterolemic effects of Lactobacillus plantarum KCTC3928 by increased bile acid excretion in C57BL/6 mice. Nutr. 2010;26(3):321-330.
17. Danielson A, Peo E, Shahani K, Lewis A, Whalen P, Amer M. Anticholesteremic Property of Yogurt Fed to Mature Boars. J. Anim. Sci. 1989;67(4): 966-974.
18. Mathara JM, Schillinger U, Guigas C, Franz C, Kutima PM, Mbugua SK, et al. Functional characteristics of *Lactobacillus spp*. from traditional Maasai fermented milk products in Kenya. Int. J. Food microbial. 2008;126(1):57-64.
19. Wang Y, Liu H, Li P, Wang YX, Xiong LX. Isolation and identification of lactic acid bacteria producing bile salt hydrolase and study on mechanism of reducing cholesterol. Food Sci. 2006;27(10):215-218.
20. Rudel LL, Morris MD. Determination of cholesterol using o-phtaldehyde. J lipid Res. 1973;14:364-366.
21. Liong MT, Shah NP. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. Journal of Dairy science. 2005;88:55-56.
22. Jiang X, Li X, Zhang B, Ren D. Isolation and identification of nitrite-degrading lactic acid bacteria from traditional pickled vegetables. Chin. Brew. 2008;1:13-16. Chinese.
23. Yan PM, Xue WT, Tan SS, Zhang H, Chang XH. Effect of inoculating lactic acid bacteria starter cultures on the nitrite concentration of fermenting Chinese paocai. Food Control. 2008;19(1):50-55. Chinese.
24. Lee J, Kim Y, Yun HS, Kim JG, Oh S, Kim SH. Genetic and proteomic analysis of factors affecting serum cholesterol reduction by Lactobacillus acidophilus A4. Appl. Environ. Microbiol. 2010;76(14): 4829-4835.
25. Choi IK, Jung SH, Kim BJ, Park SY, Kim J, Han HU. Novel Leuconostoc citreum starter culture system for the fermentation of kimchi, a fermented cabbage product. Antonie van Leeuwenhoek. 2003;84(4): 247-253.
26. Park KY, Jeong JK, Lee YE, Daily JW, 3rd. Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. J. Med. Food. 2014;17(1):6-20.
27. Li G. Intestinal probiotics: Interactions with bile salts and reduction of cholesterol. Procedia Environ. Sci. 2012;12:1180-1186. Chinese.
28. Zhang H, Huang S, Li F, Zhou H, Feng W, Li X, et al. Progress in studies of antibiotic resistance among probiotics fermented foods. Food Ferment. Ind. 2009;7:119-122. Chinese.
29. Liang MM, Zhang BL, Zhao ZL, Han JH. Study on antibiotic resistance of probiotic Lactobacillus. Hebei J. Ind. Sci. Technol. 2011;4:250-253. Chinese.

© 2018 Rehmat et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/25761