Isolation and Screening of Lactic Acid Bacteria Strains With Antibacterial Properties From The Vagina of Healthy Cows

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Abstract

Background Lactic acid bacteria with probiotic and antibacterial properties were isolated from the vagina of healthy cows. The purpose of the study is to isolation and screening of lactic acid bacteria strains with antibacterial properties from the vagina of healthy cows, which could be used to treat cow vaginal inflammation.

Results Isolation and identification of eight dominant lactic acid bacteria strains from 55 isolates was performed using classic microbiology methods and fermentation engineering. Eight strains were selected that had no spores and capsules, exhibited strong acid production capacity (pH <4.5) and had a rapid acid production (time ≤12 h) at the lowest pH. These strains were screened using fermentation engineering, pharmacology, cell biology and molecular biology methods. *Lactobacillus johnsonii* (SQ0048) had the lowest pH (4.32) and shortest acid-producing time (8 h). *L. johnsonii* (SQ0048) could produce hydrogen peroxide, inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* and adhere to the vaginal epithelial cells of cows. The average number adhering to each cell was 304±2.67. Bacteriocin genes were detected in *L. johnsonii* (SQ0048), and the bacteriocin gene of a positive clone of this strain was 100% similar to that of *Lactobacillus johnsonii* NCC 533 (NC_005362.1). Expression of the bacteriocin genes had inhibitory activity against *S. aureus* and *E. coli*.

Conclusions These advantages indicate that SQ0048 is a promising candidate for use in antimicrobial preparations.

Background

Pathogenic microorganisms can enter the uterus of postpartum cows via the vagina because of cervix expansion. This can cause endometritis and reduce reproduction and milk production [1]. Veterinary medicine usually focuses on antibiotics for treatment of reproductive tract infections. However, the continued use of antibiotics can induce bacterial resistance to antibiotics. Also, the increasing amount of drug residues has become a problem that endangers human health. Probiotics could potentially be used as biological controls to reduce antibiotics in livestock production. Probiotics are environmentally friendly and more sustainable because of the slower pace of resistance development in biological control agents.

The normal microecological environment of the human vaginal system is a complex and dynamic ecosystem inhabited by lactic acid bacteria (LAB) [2-4]. LAB are generally composed of beneficial strains that protect the vaginal environment from invasion by potentially harmful microorganisms. Beneficial LAB could improve the disorder caused by BV (bacterial vaginosis) and prevent or treat VVC (vulvovaginal candidiasis). LAB, used as probiotics, can also increase the number of immune cells and enhance immune function [1]. However, the bacterial communities in the vagina of healthy cattle are not well known. LAB dominate the vaginal flora of dairy cows under normal conditions [3]. Bacteria infecting the vagina of dairy cows mainly include *Escherichia coli* and *Staphylococcus aureus* [4]. *Ametaja. et al.* (2014) perfused LAB (*L. sakei* FUA 3089, *P. acidilactici* FUA 3140, and *P. acidilactici* FUA 3138) isolated

from the vagina of cows, and they reduced purulent vaginal secretions and improved animal health [5]. Deng *et al.* (2015) injected LAB into the vagina of cows to reduce uterine infection, regulate local and systemic immune responses and improve cow health [6]. LAB can inhibit uterine inflammation and reduce infection caused by *E. coli* and *S. aureus*. LAB can change the vaginal environment, limit the amount of pathogenic bacteria reaching the uterus and reduce the prevalence of uterine disease in cows. However, in one study, LAB did not reach the endometrium when they were injected into the vagina [7].

The method by which LAB inhibits pathogenic bacteria appears to be related to the production of lactic acid, hydrogen peroxide, bacteriocin and other active substances. It is also related to LAB adhesion and competitive adhesion to pathogenic bacteria. Lactic acid protects the body tissues by maintaining a pH value between 4 and 4.5 that creates an unsuitable environment for pathogenic microorganisms. Also, LAB can inhibit pathogenic bacteria by releasing bacteriocins and maintaining the dynamic balance of the microorganisms. Hydrogen peroxide (H_2O_2) is also a defense factor used by LAB against pathogenic microorganisms [8]. LAB shows strong adhesion to stratified non-keratinized epithelium, which can repel or inhibit pathogenic microorganisms such as *Candida albicans*, *Gardnerella vaginalis*, *E. coli* and *S. aureus* [9]. The screening process is a critical step in discovering probiotics with strong antibacterial activity. The present study focused on the screening and isolation of a LAB strain with superior antimicrobial qualities. Desirable LAB qualities include production of hydrogen peroxide, inhibition of pathogenic microorganisms, ability to adhere to vaginal epithelial cells of cows, possession of bacteriocin genes and production of high levels of acid. Our objective was to discover new LAB strains that could be used in microecological preparations for preventing and treating vaginal inflammation in cows.

Methods

Samples resource

The normal flora changes in the birth canal with cow growth. There are relatively few LABs during the juvenile period, but LAB populations significantly increase in adults. The first 10–45 months of dairy cow life are the most fertile period. Therefore, 50 healthy dairy cows aged 10–15 months without any disease were selected as experimental samples from different dairy farms in Hohhot, Inner Mongolia.

Isolation and diversity identification of the LAB strains

Isolation of the LAB strains

The LAB strains were isolated from the experimental treatments. First, mucus of the samples was prepared in PBS (phosphate buffer solution) and plated in LAPTg [10] (containing 0.2% bromocresol purple) agar pH 6.6 medium for 12–24 h at 37°C under anaerobic conditions. Then, isolated strains were picked from each plate and transferred to the same medium. The growth observed in pure cultures was recorded. Strains were freeze-dried and stored in milk yeast extract (10% (w/v) skim milk powder, 0.5% (w/v) yeast extract) at -80°C. (Strain activity degrades 40% after 1 year at room temperature.)

Identification of the LAB strains

The dominant LAB strains in this study were first screened according to Chinese Pharmacopoeia [11]. Strain morphology was selected using Gram staining. Acid-producing properties were measured using acidity testing [12]. The probiotic strains that were previously freeze-dried were tested for stability after passage by subjecting to culture characteristics, bacterial morphology, water activity and purity testing, in accordance with the requirements of the third part of the Chinese Pharmacopoeia (2010) [11].

The dominant LAB was taxonomically identified by phenotype tests. Biochemical and sugar fermentation tests were performed by an API50 CHL test strip (BioMérieux, France) according to the Bergey's Manual of Determinative Bacteriology.

The dominant LAB strains were transferred into LAPTg agar, and then incubated at 37°C under the anaerobic condition for 24 h before DNA extraction. The DNA extraction of the isolated LAB strains was done using a Bacterial Genomic DNA extraction kit (Sigma, Germany). Amplification of 16S rRNA was carried out using the forward primer 27F (5'AGAGTTTGATCCTGGCTCAG3') and the reverse primer 1492R (5'GGCTACCTTGTTACGACTT3'). The obtained single positive clones were sequenced, and then identified through the Genbank database using the BLAST algorithm. The phylogenetic status of the isolated LAB strains was established through analysis with software MEGA 5.0 version.

Acid production analysis of candidate strain

Acid production was measured using the acidity method [12]. The dominant strains identified were inoculated from stage 1 to 4 in LAPTg broth medium under anaerobic conditions. The pH of samples was taken at 0, 3, 5, 8, 10, 12, 16, and 18 h at stage 4.

Hydrogen peroxide production analysis of candidate strains

Hydrogen peroxide production was determined using the catalase test [13]. TMB (3,3', 5,5'-Tetramethyl-Benzidine) (Gibco, France) was added to 100% ethanol, heated and dissolved in a water bath, and then added to LAPTg agar medium, sterilized at 121°C for 15 min. Later, when the temperature was ~50°C, peroxidase (Gibco, France) was dissolved and added to the plate by filtering with a bacterial filter. After solidification, the plate was cultured at 37°C for 12 h. Single colonies of the dominant strains were picked to draw a line in the prepared plates and placed at 37°C for 36 h in an anaerobic environment. The plates were then taken out and left for 30 min to observe the color change of the colonies under aerobic conditions. Based on the color intensity, the strains were classified as strong (blue), medium (brown), weak (light brown) or negative (white colonies) producers of hydrogen peroxide [13].

Bacteriocins detection in candidate strains

The DNA of the strain was extracted (Sigma Bacterial Genomic DNA extraction kit (Sigma, Germany)). The primers were designed on the basis of the *Lactobacillus johnsonii* NCC 533 (NC_005362.1) sequence published on GenBank with 99% similarity to the strain. Forward Primer: 5'-GGGAATGTGACGATAATGAC-

3'; Reverse Primer: 5'-AAATCCTACTTTCTTATCTTGC-3'. The DNA was amplified and transformed. The positive clones were sequenced and ligated with the pMD19-T vector (Takara, Japan) to form a recombinant plasmid. The primers were designed again according to the BamHI and XhoI (ThermoFisher, USA) restriction sites. Forward Primer: 5'-CGCGGATCCATGAAACAATTTAATTATTTATCACA-3', Reverse Primer: 5'-CCGCTCGAGCTACTTTCTTATCTTGCCAAAA-3'. The recombinant plasmid and pET-28a (ThermoFisher, USA) were PCR amplified with primers to splice and transform. The constructed expression product was cultured in LB medium containing kanamycin at 37°C for 12 h, and the growth curve was monitored. At the pre-middle stage, the logarithmic growth of the bacterial solution was induced and cultured at 37°C for 3 h at 1 mM IPTG (Isopropyl β-D-Thiogalactoside) (Thermo, USA) [14]. Then, they were centrifuged at 6000 r/min for 10 min at 4°C. The bacteria were collected and added to a lysis buffer equivalent to 1/10 volume of the bacterial solution to resuspend. Cells of the re-suspended bacteria in the solution were broken by ultrasound. At this time, the strain of the bacterial solution was clarified. Bacteriocins gene expression was detected using SDS-PAGE. The SDS-PAGE experiment was performed in accordance with Genetic Engineering Pharmaceuticals [15]. The main reagent manufacturer: SDS-PAGE Gel Preparation Kit (Thermo, USA).

Cell adhesion testing of the candidate strain

BVECs (Primary bovine vagina epithelial cells) were cultured up to 3 passages and washed twice with DPBS before use. The LAB cultures were centrifuged at 5000 r/min for 5 mins and washed with DMEM/F12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Gibco, France)) without any additions. Cell concentration was adjusted to n × 10^8 CFU / mL, and then we added bovine vaginal epithelial cells. They were cultivated together at 37°C for 4 h in a CO_2 incubator [16]. The cell culture solution was then discarded, and Wright-Giemsa staining was performed. The cells were observed under a microscope.

The cells with the bacteria were stained with Swiss-Giemsa. Twenty fields of view were randomly selected under the microscope. The number of bacteria on 50 cells was counted, and the average number of bacteria adhering to each cell was calculated. *Lactobacillus delbrueckii* (ATCC 11842) was used as a positive control. This experiment was repeated three times.

Antibacterial susceptibility and bacteriocins detected in the candidate strain

This experiment was performed using the Oxford Cup method [17]. The methods used for evaluating the antibacterial activity of LAB against pathogenic microbial strains were described by Shokryazdan et al. [18, 19]. The bacterial liquids of LAB, *E. coli* and *S. aureus* were centrifuged, washed and adjusted to a concentration to n × 10^8 CFU/mL with PBS. One milliliter of *E. coli* or *S. aureus* was, respectively, added onto sterile plates. Then Mueller-Hinton Agar (containing 1% glucose) was poured and shaken well in sterile plates. After solidifying and drying, sterile Oxford cups were placed evenly at equal distances. A 0.2 mL amount of the LAB suspension was added into the cup and allowed to stand for 2 h, then incubated at 37°C for 36 h. The PBS phosphate buffer was used as a negative control, and streptomycin (10

μg/mL) (Batch number: 130307-201009) (Biological Product Testing Institute, China) or Penicillin G (0.12 μg/mL) (Batch number: 130437-201005) (Biological Product Testing Institute, China) [20] was used as a positive control. Vernier calipers were used to measure the diameter of the bacteriostatic zone. The judgment criteria were inhibition range <6 mm negative (-), 6–10 mm mild inhibition (+), 10–14 mm strong inhibition (++) and >14 mm very strong inhibition (+++) [18]. This experiment was repeated three times. The bacteriostatic performance of bacteriocins gene expression was tested. The LAB suspension was used ss a proxy for bacteriocin expression. Other experimental steps were the same as above. All experiments were repeated three times.

Results

Isolation and diversity identification of the LAB strains

Among the initial 55 strains, 43 strains had no spores and no capsules. These 43 LABs were in continuous culture for 30 generations according to stability requirements of the Chinese Pharmacopoeia. A total of 28 strains retained stable culture characteristics, morphology and water activity after passage. Eight strains were selected with strong acid-producing ability (pH<4.5) and a rapid acid-producing rate (time≤12 h) through acidity method requirements of the Chinese Pharmacopoeia from 28 strains. The eight strains were labelled SQ0012, SQ0015, SQ0030, SQ0041, SQ0045, SQ0048, SQ0049 and SQ0054 (Table 1).

Identification of LAB strains

The eight strains were distinguished by morphology, biochemical characteristics, sugar fermentation and 16S rRNA gene sequencing. These isolated strains were classified as follows: SQ0012 *L. plantarum*, SQ0015 *L. brevis*, SQ0030 *Enterococcus faecalis*, SQ0045 *L. kitasatonis*, SQ0054 *Lactococcus garvieae*, SQ0048 *L. johnsonii* and SQ0049 *L. amylovorus*. Based on analysis of identification results, SQ0030 and SQ0041 were the same strain. So, only the SQ0030 strain was retained (Table 2). A phylogenetic tree was constructed based on the 16S rRNA gene, which established the classification status of the seven isolated LAB strains (Fig. 1).

Screening of candidate strains

Acid production

The pH order, from high to low, was SQ0045 >SQ0030>SQ0015> SQ0054=SQ0012>

SQ0049>SQ0048 (Fig. 2). SQ0054 had the highest pH (4.5), while SQ0048 had the lowest pH (4.32). The order of the time of acid-production was SQ0015=SQ0030

>SQ0054>SQ0012=SQ0045=SQ0048=SQ0049 at the lowest pH. The acid-producing time of SQ0015 was the longest (12 h), but SQ0012, SQ0045, SQ0048 and SQ0049 had the shortest acid-producing times (8 h) at the lowest pH.

Hydrogen peroxide performance

SQ0048 had a strong positive reaction for H_2O_2 and produced a blue color, while the other strains did not change color (Fig. 3).

Bacteriocins

The bacteriocin of SQ0048 was extracted because it had better acid-producing performance and hydrogen peroxide production than the other strains. A 400 bp fragment of SQ0048 bacteriocin was detected (Fig. 4). Sequencing results showed that the positive clone strain of SQ0048 was 100% similar to *Lactobacillus johnsonii* NCC 533 (NC_005362.1). Expression of the bacteriocin gene was detected by SDS-PAGE. SQ0048 had a clear target band at about 10 kD (Fig. 5).

Adhesion of bacteria to cells

SQ0048 had obvious adhesion to the vaginal epithelial cells of cows (Fig. 6). The average number of SQ0048 adhering to each cell was 304±2.67, but the average number of *L. delbrueckii* adhering to each cell was 32±3.15. The average number of SQ0048 adhering to each cell was significantly greater than *L. delbrueckii* (P<0.01).

Antibacterial effect

SQ0048 had a weak inhibitory effect on *S. aureus* and *E. coli* (Table 3, Fig. 7). However, the expression of the bacteriocin gene of SQ0048 had very strong inhibition on *S. aureus* and *E. coli* (Table 4, Fig. 8). The antibacterial expression of the SQ0048 bacteriocin gene was significantly greater than SQ0048, the positive control group and the blank control group (PBS) (P < 0.01).

Discussion

Pathogenic microorganisms can enter the uterus from the vagina, via procreation or the environment, and cause infection. Infections have the potential to affect reproduction and milk production of cows and cause economic losses. Antibiotic treatments are typically effective at killing or inhibiting the pathogenic microorganisms that cause vaginal inflammation in dairy cows. However, the persistence of antibiotic residues in milk and beef is a human health issue. Other methods for treating cow vaginal inflammation, which minimize antibiotic use, are needed.

Lactic acid bacteria (LAB) are fundamental in the production of fermented foods and several strains are regarded as probiotics [21]. Living bacteria and metabolites can function as bacteriostatic agents but also can enhance immunity. LAB do not have drug resistance issues and are not toxic to humans or animals [22]. LAB are recognized as the dominant species in healthy vaginal microbial flora [23]. A lack of LAB is believed to disrupt the balance of vaginal microbial flora and reduce the immune barrier function. It can lead to reproductive tract inflammation, parasitic diseases, fungal and chlamydial infections [24]. Micro-ecological preparations made by LAB have previously been used in clinical practice. They have

played an important role in maintaining or adjusting the micro-ecological balance of the body and preventing diseases. However, research on the bacterial communities in the vagina of cattle is relatively limited.

The SQ0048 isolate produced the lowest pH (4.31–4.33). The acid-production times of SQ0012, SQ0045, SQ0048 and SQ0049 were 8 h at the lowest pH. SQ0048 appeared to have better acid-producing ability than the other microbial strains. SQ0048 also had the highest hydrogen peroxide-producing ability of all LAB identified. A pH value between 4 and 4.5 and hydrogen peroxide production are both strong defense factors against pathogenic microorganisms. SQ0048 was therefore used as a candidate strain and studied to determine if it possessed other antibacterial properties. The bacteriocin gene of SQ0048 was successfully cloned and expressed. SQ0048 and its bacteriocin gene expression had inhibitory activity against *S. aureus* and *E. coli*, which was consistent with the activity of other LAB bacteriocins against pathogenic bacteria [31-33]. Moreover, the strains of *L. plantarum* showed significant inhibitory activity against various fungi that commonly contaminate food indicating their potential as a bio-preservative of food [34]. The average number of SQ0048 adhered to each cell was 304±2.67, which was significantly greater than *L. delbrueckii*. SQ0048 also appeared to have strong adhesion. These factors could repel and inhibit pathogenic microorganisms [30]. Our data showed that SQ0048 has the potential to become a strain in microecological preparations used to prevent and treat inflammation of the cow vagina. Additional research will be necessary, such as safety assessment and other related research to provide sufficient technical and efficacy support for formulation development.

Conclusions

SQ0048 had antibacterial efficacy and pharmacological potential. It could potentially be used as a vaginal probiotic, through its toxicity to pathogenic microorganisms, for the prevention and treatment of bovine vaginal infections.

Abbreviations

LAB: lactic acid bacteria

PCR: polymerase chain reaction

BV: bacterial vaginosis

VVC: vulvovaginal candidiasis

IPTG: isopropyl β-D-thiogalactoside

Declarations

Ethics approval and consent to participate

All animal experiments were carried out according to the practices and standards approved by the Animal Welfare and Research Ethics Committee of Jining Normal University (Inner Mongolia, China) (Approval ID: 41, 010, 620–1), and all efforts were made to minimize animal suffering. All animals were owned by private farms and we also obtained informed consent to use the animals in our study from the owner (s) of the animals.

Consent for Publication

Not Applicable

Availability of data and material

All of the data generated or analyzed during this study are included in this published article, and the supplementary information files will be freely available to any scientist wishing to use them for non-commercial purposes upon request via e-mail with the corresponding author.

Competing interests

The authors have no conflicts of interest.

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Authors' contributions

LYM participated in the design of the study. LPF managed the study. CC, ZLC, FYR, MXH and WHX conducted data processing and operations. CC drafted, and LZG critically revised, the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Acid-producing ability of LAB isolates (n=3)

Key	Acid-producing time at the lowest pH (h)	Lowest pH ($\overline{X} \pm S$)
SQ0012	8	4.45 ± 0.01
SQ0015	12	4.47 ± 0.01
SQ0021	18	5.01 ± 0.02
SQ0022	18	5.03 ± 0.04
SQ0023	18	5.01 ± 0.02
SQ0045	8	4.50 ± 0.00
SQ0048	8	4.32 ± 0.01
SQ0049	8	4.41 ± 0.02
SQ0051	18	5.12 ± 0.01
SQ0052	12	5.07 ± 0.03
SQ0006	24	5.09 ± 0.01
SQ0018	18	5.22 ± 0.02
SQ0005	18	5.17 ± 0.02
SQ0017	18	5.13 ± 0.02
SQ0020	12	5.16 ± 0.03
SQ0024	18	5.18 ± 0.01
SQ0025	18	5.18 ± 0.02
SQ0033	12	5.14 ± 0.02
SQ0042	18	5.16 ± 0.02
SQ0028	18	5.12 ± 0.02
SQ0029	18	5.04 ± 0.01
SQ0031	18	5.21 ± 0.01
SQ0030	12	4.49 ± 0.01
SQ0039	18	5.22 ± 0.02
SQ0041	12	4.48 ± 0.00
SQ0044	18	5.32 ± 0.02
SQ0053	18	5.27 ± 0.02
SQ0054	10	4.45 ± 0.01

Table. 2 Biochemical and morphological characteristics of seven dominant LAB

Number	Similarity	Sequence number	Bacteria
SQ0012	99%	AB601168.1	*L. plantarum*
SQ0015	99%	KM392069.1	*L. brevis*
SQ0030	99%	KU353625.1	*Enterococcus faecalis*
SQ0041	99%	KU353625.1	*Enterococcus faecalis*
SQ0045	100%	AB107637.1	*L. kitasatonis*
SQ0048	99%	KU991814.1	*L. johnsonii*
SQ0049	99%	NR_075048.1	*L. amylovorus*
SQ0054	99%	KT428591.1	*L. garvieae*

Table. 3 Antimicrobial activity of the SQ0048 ($\overline{X} \pm S$, n=3)

	SQ0048 (mm)	Penicillin G (mm)	Streptomycin (mm)	PBS (mm)
Staphylococcus aureus (ATCC9144)	10 ± 0.50 (+) [a]	12 ± 0.91 (++)	-	0 (-)
Escherichia coli (ATCC11303)	8 ± 0.45 (+) [a]	-	12 ± 1.00 (++)	0 (-)

Note: the SQ0048 with inhibition zone < 6 mm, 6–10 mm, 11–14 mm and >14 mm was classified as negative (-), mild (+), strong (++) and very strong (+++) inhibition, respectively. A was the extremely significant difference compared with the blank. (P <0.01).

Table. 4 Antimicrobial activity of SQ0048 bacteriocin in different conditions (n=3, $\overline{X} \pm SD$)

Group	Diameter (mm) (n=3, $\overline{X} \pm SD$)	
	Staphylococcus aureus (ATCC9144)	*Escherichia coli* (ATCC11303)
SQ0048	10 ± 0.23 (+)	8 ± 0.51 (+)
Expression of bacteriocins gene of SQ0048	18 ± 0.45 (+++) [a**]	15 ± 0.60 (+++) [a**]
Penicillin G	12 ± 0.2 (++)	-
Streptomycin	-	12 ± 0.21 (++)
PBS	0 (-)	0 (-)

Note: The SQ0048 with inhibition zone < 6 mm, 6–10 mm, 10–14 mm and > 14 mm were classified as negative (-), mild (+), strong (++) and very strong (+++) inhibition, respectively; each value represents the mean value standard deviation (SD) from three trials; a was the extremely significant difference compared with the blank (P <0.01);*: The significant difference of antibacterial diameter was compared

between expression product of SQ0048 bacteriocins and antibiotics.*: The significant difference (*P<0.05*),**: The extremely significant difference (*P* < 0.01).

Figures

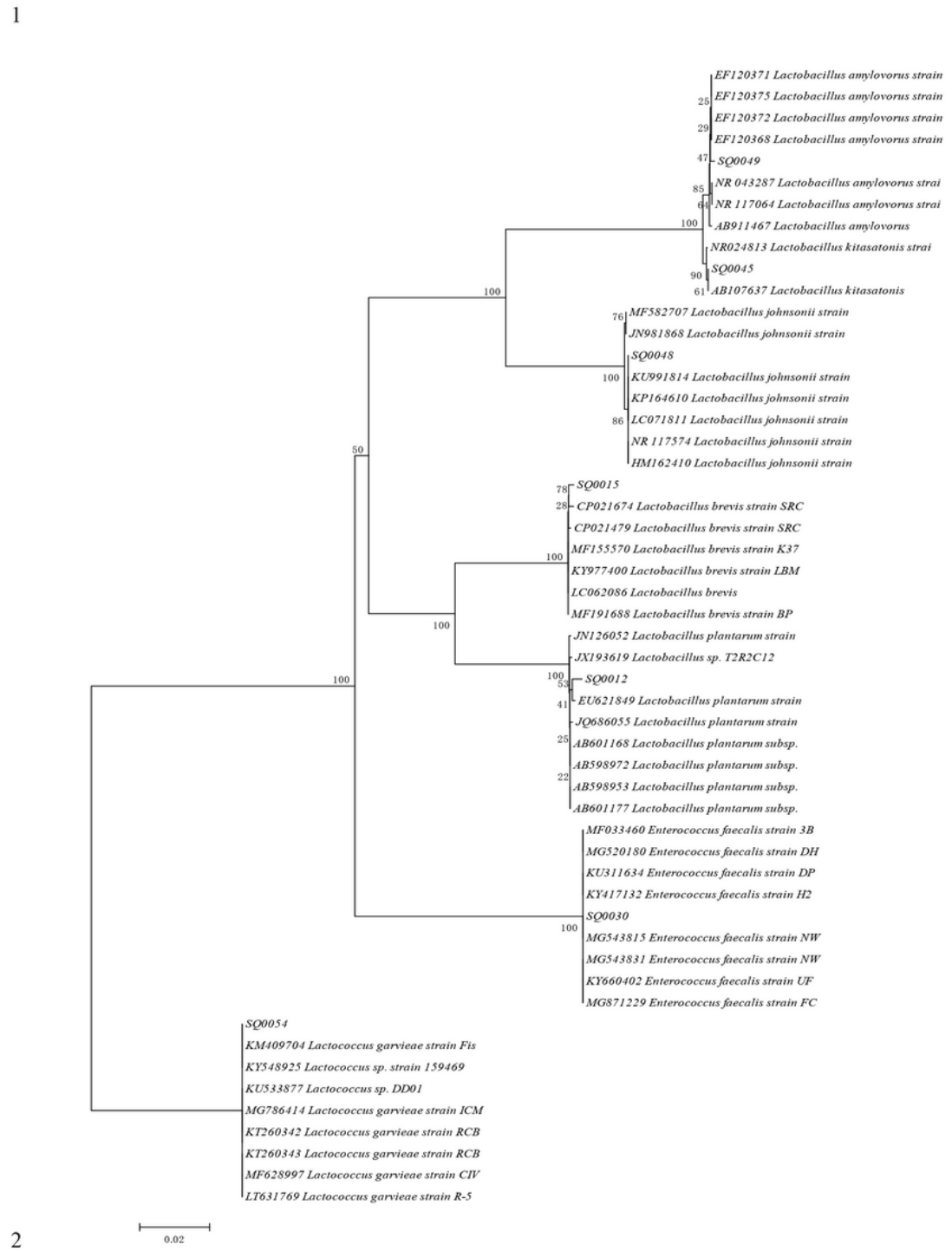


Figure.1 The constructed phylogenetic tree of the dominant LAB isolated based on the 16s rRNA gene

Figure 1

Phylogenetic tree of the dominant LAB isolated based on the 16s rRNA gene

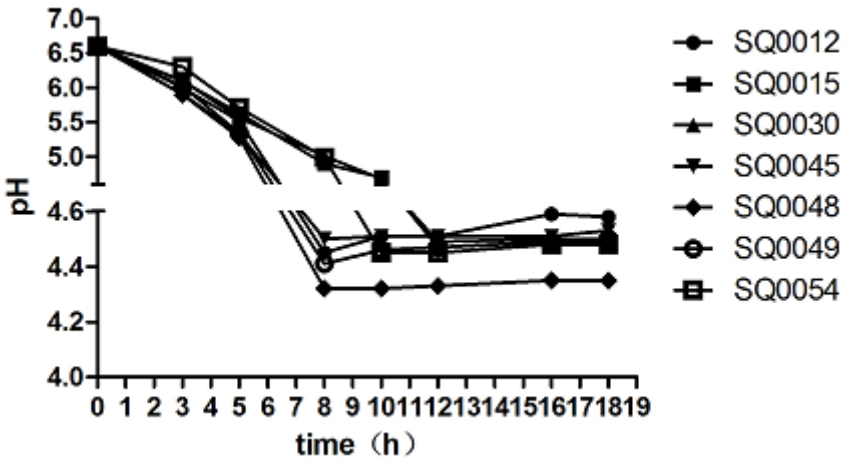


Figure 2

Acid-producing ability of the dominant LAB isolated

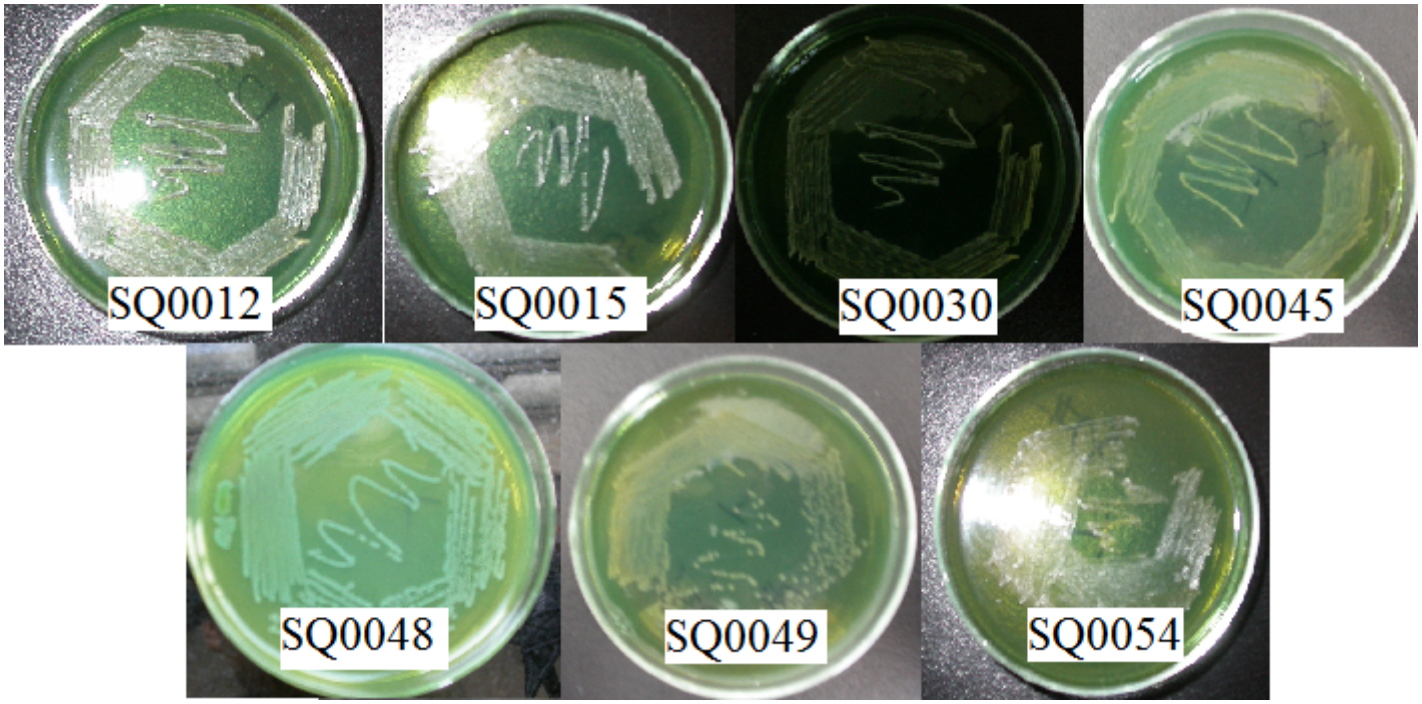


Figure 3

Production of hydrogen peroxide by the LAB strains isolated

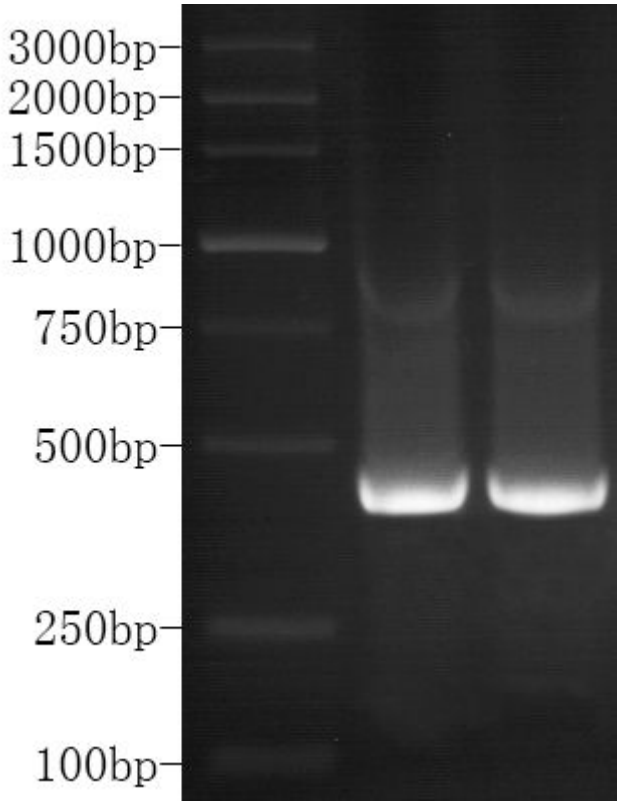


Figure 4

PCR product of SQ0048

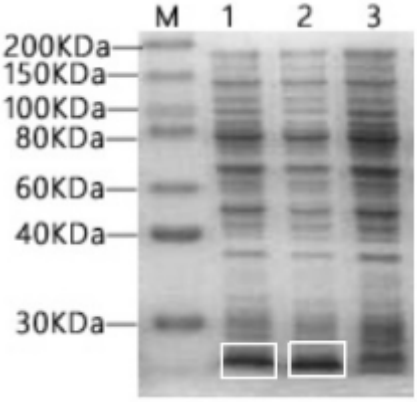
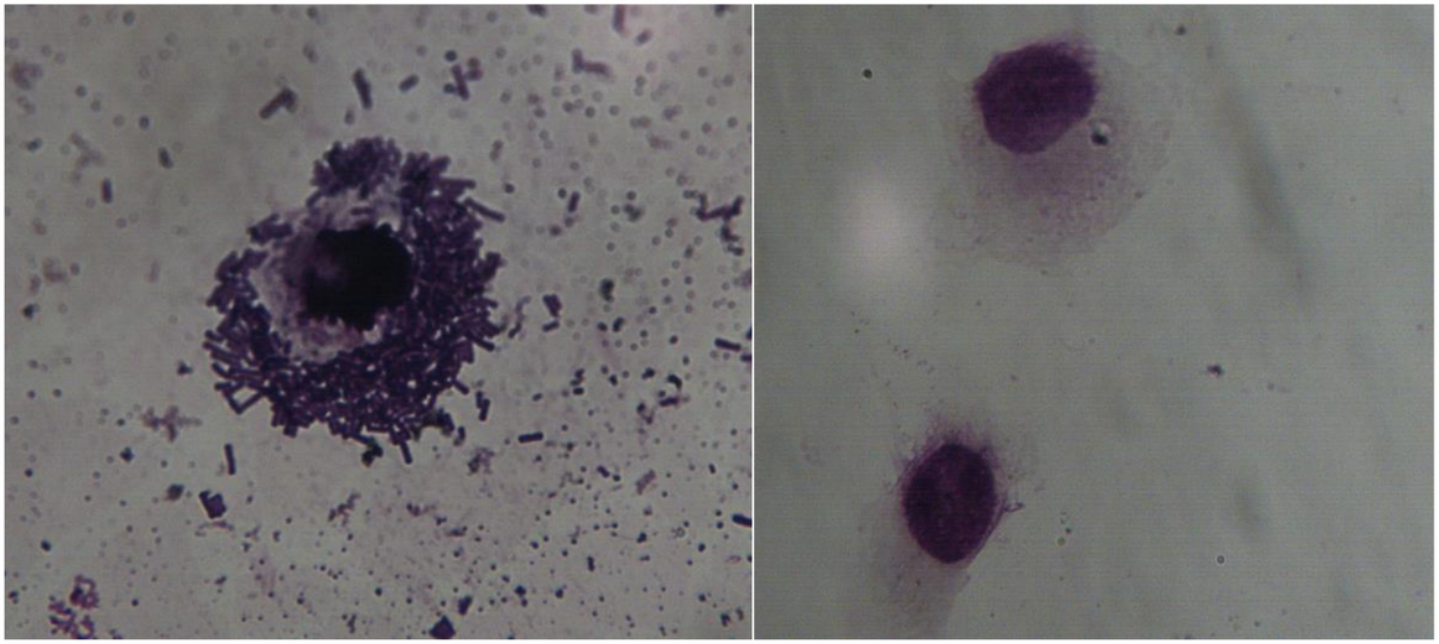
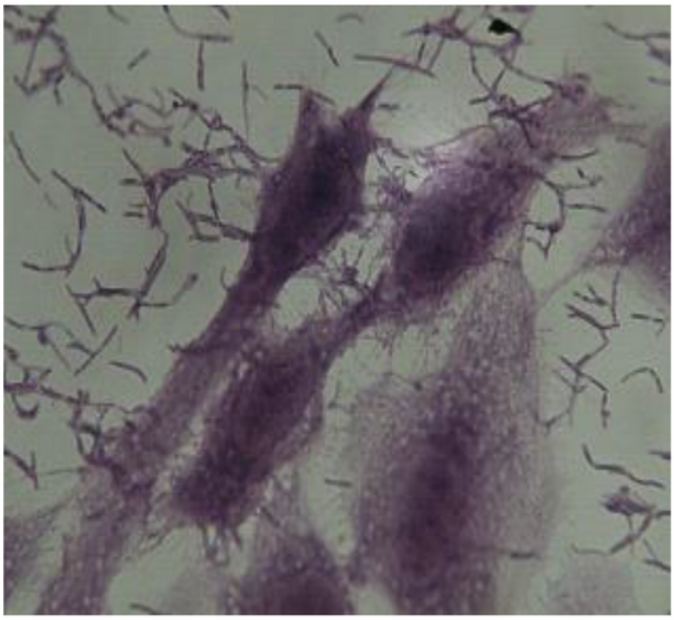


Figure 5

Results of SDS-PAGE fusion protein (Note: 1 and 2 showed the expression of bacteriocins gene of SQ0048; 3: pET28a).



A B



C

Figure 6

Results of SQ0048 adhesion to BVECs (Note: A:SQ0048; B: BVECs (blank)).

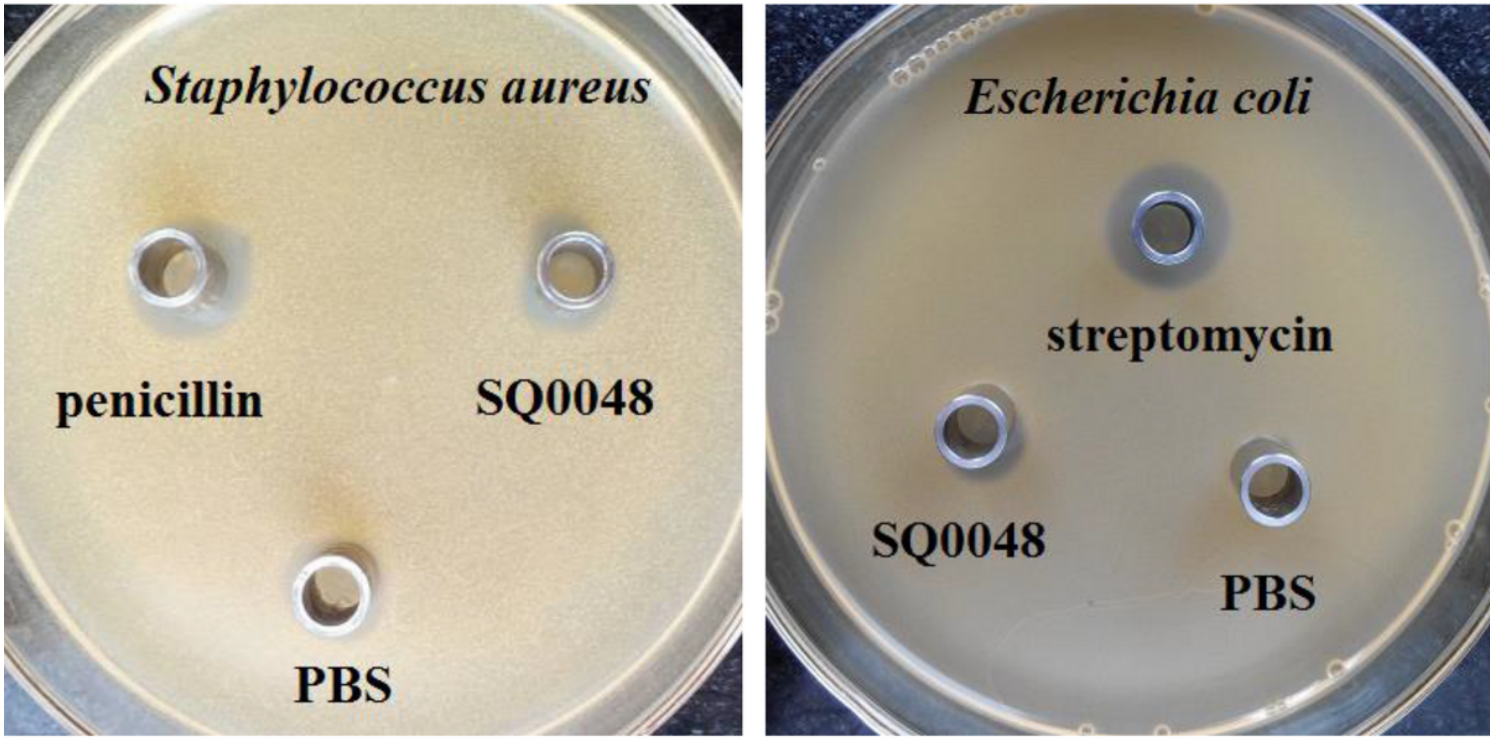


Figure 7

The antimicrobial activity of the SQ0048. Note: A: The SQ0048 strains inhibited the growth of Staphylococcus aureus; B: The SQ0048 strains inhibited the growth of Escherichia coli

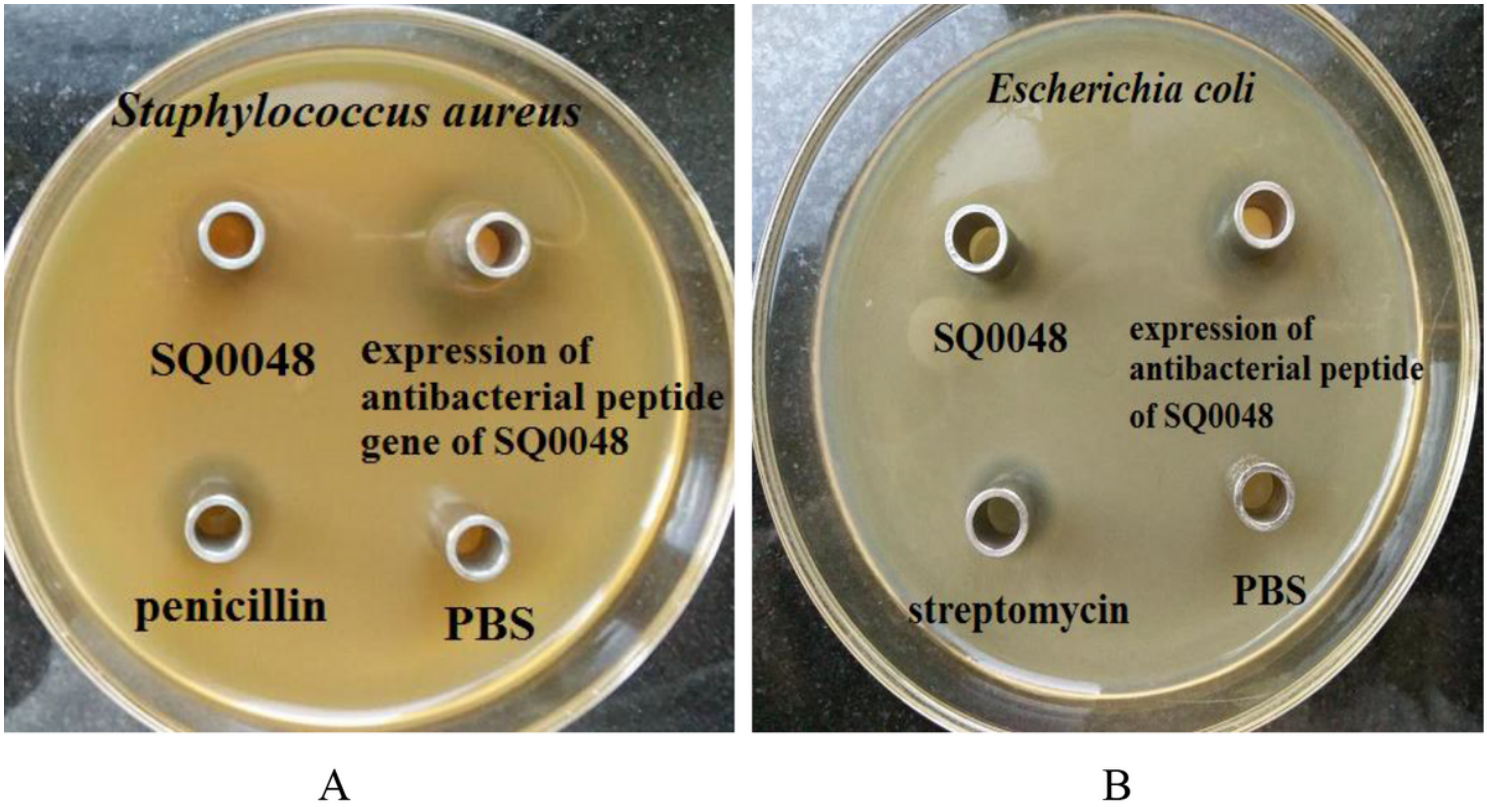


Figure 8

The antimicrobial activity of the expression product of SQ0048 bacteriocins. Note: A: The expression product of SQ0048 bacteriocins inhibited the growth of Staphylococcus aureus; B: The the expression product of SQ0048 bacteriocins inhibited the growth of. Escherichia coli.