

Isolation and Identification of Lactic Acid Bacteria Inhibiting *Staphylococcus aureus*

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Abstract: To screen lactic acid bacteria with bacteriostatic action from healthy chicken intestines, and determine its antibacterial effect against *Staphylococcus aureus*, the lactic acid bacteria against *S. aureus* were screened by double-layer plate method. The antibacterial effect was determined by Oxford Cup method. The active ingredient was identified by a 16s rDNA molecular marker to construct a phylogenetic tree. Eighteen lactic acid bacteria were screened from healthy chicken intestines. Two of them had good antibacterial effects against *S. aureus*, *Salmonella choleraesuis*, *Escherichia coli*, *Shigella*, *Salmonella*, *Salmonella enteritidis*, and *S. Enteritidis* subspecies. Two strains of lactic acid bacteria were identified as *Lactobacillus plantarum* and *Lactococcus* genus. *L. plantarum* strain and *Lactococcus* strain isolated from healthy chicken intestinal tract showed certain inhibitory effects on *S. aureus* and other key pathogenic bacteria.

1. Introduction

S. aureus is a representative strain of Gram-positive bacteria [1]. It is abundant in nature and has the title of “felophilic bacteria”. It is an important zoonotic pathogen that can cause food poisoning and suppuration, infection, pneumonia, pericarditis, lethal shock and other diseases [2-4]. Among the series of diseases caused by bacteria in various countries, diseases infected by *S. aureus* rank second [5]. On the other hand, in order to prevent and treat *S. aureus*, a large number of antibiotics have been used, leading to the emergence of multi-drug resistant strains, such as methicillin-resistant *S. aureus*, which is now widely distributed in hospitals, all kinds of quick-frozen rice noodles, barbecues, food, raw meat and other foods, and the environment have great difficulties in prevention and control, which has aroused great concern from all walks of life [6, 7].

Lactic acid bacteria (LAB) is a kind of bacteriostatic polypeptide or protein substance that is synthesized and secreted into the environment by ribosome mechanism during metabolism [8, 9]. It can be degraded in human body and has high efficiency, no drug resistance and no toxicity. With no residue, it has become a hot spot in the research and development of natural food biopreservatives. In recent years, the inhibitory effect of LAB on pathogenic bacteria in meat products has attracted wide attention of researchers, and the mechanism of inhibition has also been clarified [10-15]. However, the development of LAB that have inhibitory effects on pathogenic bacteria is still very scarce due to its late attention, and the number of species and application range are limited, especially in the upstream of food production (such as poultry, eggs, meat). This study is to screen LAB with significant inhibition of *S. aureus* from the intestinal tract of healthy chickens, and then analyze the antibacterial substances, and identify them by molecular biology. The results will provide useful LAB resources for the control of *S. aureus* in pork food production.

2. Materials and methods

Directly take the healthy chicken intestines of the Wuliangshan Silky Chicken Farm in Nanxun, Yunnan Province, and we wash the chicken intestines with sterile saline and scrape the contents of the intestines, scrape the mucus at different positions on the surface of the intestinal mucosa, and

place them in liquid Manne-Rogosae-Sharpe (MRS) culture. The medium was incubated at 37 °C for 12 hours. 100 µL of the bacterial solution was applied to MRS solid medium plate (containing 5% CaCO₃), and cultured at 37 °C for 24 h [16, 17]. Single colonies with the strongest growth and clear calcium lysate were selected and purified on MRS solid medium. The colonies that produced the calcium lysate were subjected to Gram staining and catalase experiments, and Gram staining was selected. The positive, catalase-negative strains were initially identified as LAB, and the above strains were stored in a -80 °C refrigerator in MRS medium containing 15% glycerol [18, 19].

Using LAB genomic DNA as a template, 16S rDNA gene of LAB was subjected to PCR amplification using universal primers 27F (5'-AGAGTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGATACCTTGTACGACTT-3'). The PCR amplification procedure was: pre-denaturation at 94 °C for 5 min; then denaturation at 94 °C for 30 S, annealing at 52 °C for 30 S, extension at 72 °C for 50 S, 35 cycles; extension at 72 °C for 10 min. The size of the PCR product was verified by agarose gel electrophoresis, and the target band was purified, and then sent to Bioengineering (Shanghai) Co., Ltd. for Sanger sequencing of the PCR product.

3. Results and discussion

The LAB in the chicken intestine were screened by MRS medium containing Ca₂CO₃, and cultured in a 37 °C incubator for 24 h to select colonies with strong growth and obvious calcium-dissolved circles. The colonies were continuously streaked on a MRS solid medium plate to obtain 18 strains of Gram-positive bacteria suspected to have a lytic cycle, and 29 strains of suspected LAB, numbered L1 to L29. In this study, two strains of LAB with good antibacterial activity against *S. aureus* were isolated from the intestinal tract of healthy adult Wuliangshan black bone chicken. According to the 16S rDNA sequence analysis, two LAB strains with bacteriostatic action were identified as *L. plantarum* and *Lactococcus*.

At present, food production and processing are developing in the direction of no antibiotics and zero food safety issues. Lactic acid bacteria can not only inhibit bacteria, but also improve flavor and strengthen intestinal micro-ecological balance. The lactic acid bacteria that inhibit *S. aureus* in this study are valuable probiotics, and can reduce the risk of food poisoning caused by pathogenic bacteria in food. In addition, *L. plantarum* and *Lactococcus* also provide valuable strain resources for further research and the development of antibiotic substitute products and foods without non-toxic side effects.

4. Summary

These two strains showed a certain inhibitory efficiency for various key pathogenic bacteria, and they will be useful probiotic resources in food control in the future. However, their roles in actual production are still need to be confirmed.

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References

- [1] J.M. Ling, A.W. Lam, E.W. Chan *et al.*, What have we learnt from community-acquired infections in Hong Kong?, *J. Anti. Chem.* 51 (2003) 895.
- [2] Z. Zhou, M. Zhang, H. Li *et al.*, Prevalence and molecular characterization of *Staphylococcus aureus* isolated from goats in Chongqing, China, *BMC Vet. Res.* 13 (2017) 352.
- [3] D.E. Moormeier, K.W. Bayles, *Staphylococcus aureus* biofilm: a complex developmental organism, *Mol. Microbiol.* 104 (2017) 365-376.

- [4] T. Nakatsuji, T.H. Chen, S. Narala *et al.*, Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis, *Sci. Tran. Med.* 9 (2017) eaah4680.
- [5] V. N. Chaitra, Pradeep, A.N. Grace *et al.*, Electrodeposition and characterization of polyaniline films for the detection of *Staphylococcus Aureus* bacteria in food products, *Sensor Let.* 15 (2017) 65-70.
- [6] B. Zheng, H. Xu, A. Li *et al.*, Community-associated meticillin-resistant *Staphylococcus aureus* pneumonia in China, *Lancet Infect. Dis.* 17 (2017) 26.
- [7] K.A. Jackson, M.K. Bohm, J.T. Brooks *et al.*, Invasive methicillin-resistant *Staphylococcus aureus* infections among persons who inject drugs - six sites, 2005-2016, *Morb. Mort. Weekly Rep.* 67 (2018) 625-628.
- [8] S.D. Todorov, J.G. LeBlanc, B.D.G.M. Franco, Evaluation of the probiotic potential and effect of encapsulation on survival for *Lactobacillus plantarum* ST16Pa isolated from papaya, *World J. Microbiol. Biotechnol.* 28 (2012) 973-984.
- [9] N. Hwanhlem, T. Ivanova, V. Biscola *et al.*, Bacteriocin producing *Enterococcus faecalis* isolated from chicken gastrointestinal tract originating from Phitsanulok, Thailand: Isolation, screening, safety evaluation and probiotic properties, *Food Control* 78 (2017) 187-195.
- [10] S.J. Crowley, Mahony, D. van Sinderen, Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives, *Trends in Food Sci. Technol.* 33 (2013) 93-109.
- [11] D.G. Cizekiene, Juodeikiene, A. Paskevicius *et al.*, Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread, *Food Control* 31 (2013) 539-545.
- [12] J.V. Farizano, E. Masias, F. F. Hsu *et al.*, PrfA activation in *Listeria monocytogenes* increases the sensitivity to class IIa bacteriocins despite impaired expression of the bacteriocin receptor, *B. B. A.* 1863 (2019) 1283-1291.
- [13] S. Cao, R. Du, F. Zhao *et al.*, The mode of action of bacteriocin CHQS, a high antibacterial activity bacteriocin produced by *Enterococcus faecalis* TG2, *Food Control* 96 (2019) 470-478.
- [14] V. Juturu, J.C. Wu, Microbial production of bacteriocins: Latest research development and applications, *Biotechnol. Adv.* 36 (2018) 2187-2200.
- [15] M.L. Chikindas, R. Weeks, D. Drider *et al.*, Functions and emerging applications of bacteriocins, *Cur. Opin. Biotechnol.* 49 (2018) 23-28.
- [16] H.A. Sakandar, S. Kubow, F.A. Sadiq, Isolation and in-vitro probiotic characterization of fructophilic lactic acid bacteria from Chinese fruits and flowers, *LWT- Food Sci. Technol.* 104 (2019) 70-75.
- [17] O.K. Pringsulaka, O. Rueangyotchanthana, N. Suwannasai *et al.*, In vitro screening of lactic acid bacteria for multi-strain probiotics, *Liv. Sci.* 174 (2015) 66-73.
- [18] T. Nishino, Y. Matsuda, Y. Yamazaki, Separation of viable lactic acid bacteria from fermented milk, *Heliyon*, 4 (2018) e00597.
- [19] Y.Y. Hor. M.T. Liong, Use of extracellular extracts of lactic acid bacteria and bifidobacteria for the inhibition of dermatological pathogen *Staphylococcus aureus*, *Dermatologica Sinica* 32 (2014) 141-147.