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Beneficial effects of sour pork fermented with a *Lactobacillus plantarum* strain on lowering blood lipids in mice

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Abstract

As a type of main meat food, pork is widely accepted, and has been used over a long time by residents in China. However, its high cholesterol is the primary unfavourable factor that influences human health. The reduction of cholesterol content in pork through fermentation using probiotics may be a promising candidate approach. In the present work, the probiotic strains were obtained from traditional naturally fermented foods, and identified *via* monoclonal culture, morphological observation, biochemical detection, and 16S rRNA sequencing. The dominant strain was used to produce sour pork, which was applied to regulate lipid metabolism in mice. Results showed that a total of 31 strains were identified from the traditional naturally fermented foods, of which four strains had similar characteristics to *Lactobacillus plantarum*, and displayed good capacity for cholesterol degradation with metabolism efficiencies of 75.31% (DC2 strain), 68.01% (PC1 strain), 60.49% (SC1 strain), and 58.02% (DC1 strain). The dominant strain DC2 had 99.93% homology with *L. plantarum*. Following fermentation with DC2 strain (4×10^6 CFU/g inoculum size, 20-day incubation), the cholesterol in sour pork was significantly reduced with 77.2% efficiency. The serum lipids in mice that were fed with sour pork were significantly lower than those in mice that were fed with normal pork. In summary, a *L. plantarum* strain with good capacity for cholesterol degradation was obtained (CCTCC NO: M 2019121). The strain can be used to produce fermented sour pork. Dietary sour pork may be beneficial for lowering blood lipid levels.

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Introduction

Pork has long been a fundamental and important meat for production and consumption, accounting for 60% of the total meat, to more than 50 million tons of annual consumption in China (Zhu and Ma, 2020). The per capita annual consumption of pork has exhibited a steady growth trend from 12.5 kg in 1995 to 21.0 kg in recent years with changes in lifestyle (Lu and Xiao, 2020). Pork is the primary food source of animal proteins (21.4 g/100 g pork) and lipids (3.5 g/100 g pork). Pork quality closely affects resident health (Song *et al*., 2020). According to the "Chinese Dietary Guidelines", healthy lipid intake is recommended for approximately 25.0 – 30.0% of total energy (CSN, 2022). However, over 67.9% of the residents were shown to have an excessive amount of lipid intake at more than 30% (Gu *et al*., 2019; Herforth *et al*., 2019). Excess cholesterol is an important inducer of human cardiovascular and metabolic diseases, such as obesity and diabetes (Guo *et al*., 2018). Therefore, the reduction of cholesterol content *via* in-depth processing is one of the important ways to improve the availability of nutrients in pork for a healthy human diet.

There are various types of pork processed products including fermented, dried, roasted, and marinated pork with a long history of usage in China. High-temperature technology is the predominant method in pork processing. Low-temperature fermentation using probiotics is a promising approach

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to produce sour pork with unique flavour, texture, colour, and low cholesterol content for balancing dietary nutrition that is conducive for digestion and absorption (Stavropoulou *et al*., 2018). However, there are many difficulties in the pork fermentation industry, such as lack of usable probiotic resources and corresponding fermentation technology. In the present work, to obtain a fermented strain and to explore viable processing conditions of sour pork, Man Rogosa Sharp (MRS) selective cultural medium was used to isolate *Lactobacillus plantarum* strains from traditional naturally fermented foods, including *douchi*, pickled Chinese cabbage, pickled radish, and fermented pepper. The obtained isolates were identified through monoclonal culture, morphological observation, biochemical detection, and 16S rRNA sequencing. Furthermore, the fermentation characteristics were investigated under laboratory conditions. These explorations would provide a theoretical basis and experimental evidence for the industrial production of fermented sour pork.

Materials and methods

Bacterial screening culture

Four traditional naturally fermented foods namely *douchi*, pickled Chinese cabbage, pickled radish, and fermented pepper obtained from a market in Hunan province (each for 25.0 g) were incubated with 225 mL of saline solution, followed by shattering and gauze filtering procedures. The collected filtrate was serially diluted (approximate 10^{-1}-10^{-6}) with a 10-fold gradient, in which the containing bacteria were monoclonal cultured in MRS conditional medium in a humidified atmosphere at 37°C for 72 h. A typical single colony with a calcium-dissolving ring was selected, and further sub-cultured for three passages (each passage for 24 h) to obtain a pure strain.

Bacterial characterisation

The characteristics of the isolates were analysed *via* morphological observations. Bacterial cells were analysed using Gram-staining. Biochemical tests were performed using catalase, gelatine liquefication, nitrate reduction, H_2S production, glucose acid and gas production, exercise, litmus milk, and sugar fermentation tests following the China National Standard Program (Ling and Dong, 1998; Gusils *et al*., 2004).

Phylogenetic analysis

RNA extraction and 16S rRNA gene amplification and sequencing were performed by Sangon Co. Ltd. (Shanghai). The sequence was analysed by using online tools of NCBI BLAST (https://www.ncbi.nlm.nih.gov/). One hundred 16S rRNA sequences of highly homologous species from the same order as the new species were retrieved and downloaded from NCBI. Complete alignment was performed using ClustalX software with default parameters. The evolutionary history was inferred using the maximum likelihood method based on the Tamura-Nei model within MEGA6 software (Tamura and Nei, 1993; Tamura *et al*., 2013).

Investigation of cholesterol degradation efficiency

The dominant strain was inoculated into selective liquid MRS medium and cultured with shaking at 37°C for 24 h. To completely saponify fatty acids, the collected culture media (0.5 mL) at 0 and 24 h were added to anhydrous ethanol (3.0 mL) and 50% KOH (2.0 mL), and incubated at 65°C for 1 h, followed by the addition of 5% sodium chloride (3 mL) and *n*-hexane (10 mL) for the extraction of cholesterol. The *n*-hexane upper layer (2 mL) was evaporated to dryness, followed by the addition of acetic acid (4 mL). Subsequently, the cholesterol content in extracted samples or reference standards of the serial dilution was determined following the method in "Determination of Cholesterol in Foods of National Food Safety Standard (GB 5009.128-2016)" (FDAC, 2016). The standard curve with the regression equation was plotted on the linear relation between cholesterol content and absorption value at OD 560 nm. The cholesterol content in the extracted samples was determined using the regression equation. Afterwards, the cholesterol degradation efficiency was analysed as: efficiency (%) = (content at 0 h - content at 24 h)/content at 0 h (Bales *et al*., 1998).

Bacterial proliferation at extreme temperature

The bacterial cells from the dominant strain were inoculated into MRS medium, and shock cultured at 10, 37, or 45°C. The bacterial concentration was successively determined with an interval of 3 to 42 h. The proliferation curve was plotted on the linear relationship between culture time and absorption value at OD 600 nm.

Resistance of bacterial cells to acid/base environment

The dominant bacterial cells were inoculated into buffer solutions of pH 2.2, 3.6, 5.4, 7.2, 8.4, 9.6, or 10.8, and were held at 4°C. The number of active bacterial cells was analysed *via* the plate counting method using MRS medium at 0, 1, 2, 3, 4, and 8 h.

Fermentation conditions of sour pork

The pork was shaved, cleaned, cooked with boiling water, drained, and sliced. The pork was fermented using dominant bacteria with an inoculum density of 0, 1×10^6, 2×10^6, 4×10^6, 8×10^6, 16×10^6, or 32×10^6 CFU/g, followed by thorough mixing with salt, maize, and ground pepper. The mixture was loaded and sealed into a crock, and fermented for 20 d. The pork before and after fermentation was evaluated for cholesterol content using the abovementioned method.

Animal experiments and analysis of blood index

All of the animal experiments were approved by the Experimental Animals Ethical and Welfare Committee of Central South University (approval no.: 2020sydw0201). Ten female C57BL/6 mice (Hunan SJA Laboratory Animal Co., Ltd) of 7-week age were randomly allocated into one of two groups of five each, including the pre-fermentation pork group (Pre-FM) and the post-fermentation pork group (Post-FM). Additionally, they were housed in a clean room with free access to food and water. After an adaptation period of 3 d, mice in the two groups were fed pre- and post-fermentation pork. The mice were weighed every 3 d. After 15 d, the mice were euthanised to collect blood samples. The blood was analysed for total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TGs), alkaline phosphatase (ALP), glutamic-oxalacetic transaminase (AST), glutamic-pyruvic transaminase (ALT), total protein (TP), glucose (GLU), and serum lipopolysaccharide-binding protein (LBP) using a biochemical autoanalyser (DS5J0002, Sinnowa Medical Science and Technology Co. Ltd., Nanjing, China).

Statistical analysis

Data were presented as mean ± standard deviation of at least three independent experiments. The variance between all of the groups was assessed using an ANOVA, and the statistical significance of the differences between the groups was assessed using Tukey's multiple comparisons test. Statistical analysis was performed using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA). Furthermore, $^{**}p < 0.01$ and $^{*}p < 0.05$ were considered to be statistically significant.

Results and discussion

The dominant strain was Lactobacillus plantarum

There were 31 strains that were obtained *via* screening in MRS medium, in which four strains had typical characteristics to degrade cholesterol, and were similar to *Lactobacillus*, including SC1, DC1, DC2, and PC1 (Table 1).

The typical dominant strain of DC2 was further analysed *via* monoclonal culture, morphological observation, biochemical detection, and 16S rRNA sequencing. The colony with a diameter of 1.5 ± 1 mm exhibited characteristics of circular shape, swelling, white colour, and irregular edge, and changed to light pink in colour (Figure 1A). The bacterial cells exhibited blue budless and stout Gram-positive bacillus (Figure 1B). The DC2 strain displayed positive characteristics in the gelatine liquefication, glucose acid and gas production, exercise, litmus milk, and sugar fermentation tests. In addition, they showed negative characteristics in the catalase, nitrate reduction, and H_2S production tests (Table 2).

Table 1. Colony morphology of dominant isolates, and efficiency of degrading cholesterol.

Serial name	Colony morphology	Efficiency of degrading cholesterol
SC1	Circle, white, swelling, neatly edge, smooth surface, diameter 2 ± 1 mm	60.49%
DC1	Circle, white, swelling, neatly edge, smooth surface, diameter 2 ± 1 mm	58.02%
DC2	Circle, grey white turn to light pink, swelling, irregular edge, rough surface, diameter 1.5 ± 1 mm	75.31%
PC1	Circle, gloss white, swelling, neatly edge, smooth surface, diameter 1.5 ± 1 mm	68.01%

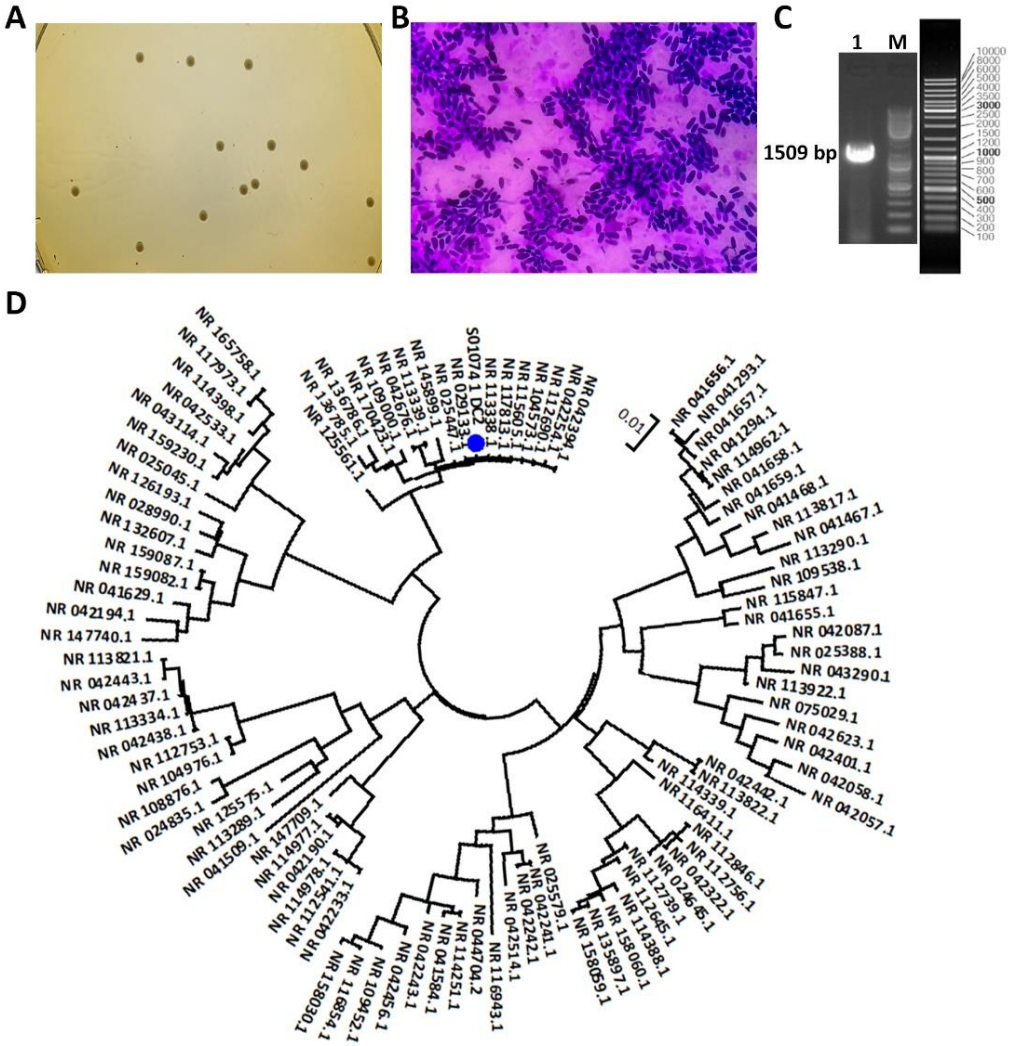

Figure 1. DC2 strain was identified as *Lactobacillus plantarum* (CCTCC No. M2019121). **(A)** Colony of DC2 strain; **(B)** Gram-staining and observation of bacterial cell morphology; **(C)** PCR amplification of 16S rRNA; and **(D)** molecular phylogenetic analysis by the maximum likelihood method and tree with the highest log likelihood (-9870.6765).

Table 2. Biochemical properties of DC2 strain.

Biochemical detection	Catalase test	Gelatine liquefication test	Nitrate reduction test	H_2S production test	Glucose acid and gas production test	Exercise test	Litmus milk test	Sugar fermentation test
DC2	-	+	-	-	+	+	+	+

Note: (+) indicates positive results, and (-) indicates negative results.

The typical dominant strain of DC2 was further analysed *via* monoclonal culture, morphological observation, biochemical detection, and 16S rRNA sequencing. The colony with a diameter of 1.5 ± 1 mm exhibited characteristics of circular shape, swelling, white colour, and irregular edge, and changed to light pink in colour (Figure 1A). The bacterial cells exhibited blue budless and stout Gram-positive bacillus (Figure 1B). The DC2 strain displayed positive characteristics in the gelatine liquefication, glucose acid and gas production, exercise, litmus milk, and sugar fermentation tests. In addition, they showed negative characteristics in the catalase, nitrate reduction, and H_2S production tests (Table 2).

A band with a size of 1509 bp (Figure 1C) was amplified from the 16S rRNA of the DC2 strain, and its sequence was as follows: DC2 (S01074-1, 1509

bp): CATGGCTCAGGACGAACGCTGGCGGCGT
GCCTAATACATGCAAGTCGAACGAACTCTG
GTATTGATTGGTGCTTGCATCATGATTTACA
TTTGAGTGAGTGGCGAACTGGTGAGTAACA
CGTGGGAAACCTGCCCAGAAGCGGGGGATA
ACACCTGGAAACAGATGCTAATACCGCATA
ACAACTTGGACCGCATGGTCCGAGCTTGAA
AGATGGCTTCGGCTATCACTTTTGGATGGTC
CCGCGGCGTATTAGCTAGATGGTGGGGTAA
CGGCTCACCATGGCAATGATACGTAGCCGA
CCTGAGAGGGTAATCGGCCACATTGGGACT
GAGACACGGCCCAAACTCCTACGGGAGGCA
GCAGTAGGGAATCTTCCACAATGGACGAAA
GTCTGATGGAGCAACGCCGCGTGAGTGAAG
AAGGGTTTCGGCTCGTAAAACTCTGTTGTTA
AAGAAGAACATATCTGAGAGTAACTGTTCA
GGTATTGACGGTATTTAACCAGAAAGCCAC
GGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTGTCCGGATTTATT
GGGCGTAAAGCGAGCGCAGGCGGTTTTTTA
AGTCTGATGTGAAAGCCTTCGGCTCAACCGA
AGAAGTGCATCGGAAACTGGGAAACTTGAG
TGCAGAAGAGGACAGTGGAACTCCATGTGT
AGCGGTGAAATGCGTAGATATATGGAAGAA
CACCAGTGGCGAAGGCGGCTGTCTGGTCTGT
AACTGACGCTGAGGCTCGAAAGTATGGGTA
GCAAACAGGATTAGATACCCTGGTAGTCCAT
ACCGTAAACGATGAATGCTAAGTGTTGGAG
GGTTTCCGCCCTTCAGTGCTGCAGCTAACGC
ATTAAGCATTCCGCCTGGGGAGTACGGCCGC
AAGGCTGAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCTACGCGAAGAACCTTACCAGGTCT
TGACATACTATGCAAATCTAAGAGATTAGAC
GTTCCCTTCGGGGACATGGATACAGGTGGTG
CATGGTTGTCGTCAGCTCGTGTCGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCT
TATTATCAGTTGCCAGCATTAAGTTGGGCAC
TCTGGTGAGACTGCCGGTGACAAACCGGAG
GAAGGTGGGGATGACGTCAAATCATCATGC
CCCTTATGACCTGGGCTACACACGTGCTACA
ATGGATGGTACAACGAGTTGCGAACTCGCG
AGAGTAAGCTAATCTCTTAAAGCCATTCTCA
GTTCGGATTGTAGGCTGCAACTCGCCTACAT
GAAGTCGGAATCGCTAGTAATCGCGGATCA
GCATGCCGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGCCCGTCACACCATGAGAGTTT
GTAACACCCAAAGTCGGTGGGGTAACCTTTT
AGGAACCAGCCGCCTAAGGTGGGACAGATG
ATTAGGGTGAAGTCGTAAA.

Via the analysis, the DC2 strain, which has an evolutionary history with the highest log likelihood (-9870.6765) involving 101 nucleotide sequences, showed close homology (99.93%) with a *L. plantarum* strain (NR_115605.1) (Figure 1D). Therefore, the DC2 strain was identified as a *L. plantarum*, and was preserved in the China Center for Type Culture Collection (CCTCC No. M2019121, Wuhan University).

DC2 strain was able to degrade cholesterol

The built standard curve had a linear relationship with a regression equation of $y = 2.4592x - 0.0018$ ($R^2 = 0.9958$) (Figure 2A). After fermentation, the sour pork displayed good texture and colour (Figure 2B) with a significant degradation of cholesterol content (77.2%) (Figure 2C). The efficiency of cholesterol degradation demonstrated a nonlinear relationship of a regression equation of $y = 0.0091x^2 - 0.1196x + 0.4769$ ($R^2 = 0.9971$) with inoculum size (Figure 2D), thus suggesting a suitable inoculum size of approximately 10^7 CFU/g. The DC2 strain proliferated slowly at 10°C, and quickly at 37 and 45°C. However, they also quickly aged at 45°C after 27 h (Figure 2E), thus suggesting the best proliferation conditions at 37°C and best storage conditions below 10°C, hence suitable for low temperature fermentation. Moreover, the DC2 strain displayed good survivability in pH 3.6, 5.4, 7.2, and 8.4 buffer solutions. However, the DC2 strain quickly died in the pH 2.2, 9.6, and 10.8 buffer solutions (Figure 2F), which suggested a better resistance of the DC2 strain to acidic conditions than basic conditions.

Low-temperature fermentation using the DC2 strain may be a promising approach to produce sour pork that can ameliorate flavour, decrease cholesterol content in pork, and improve the bioavailability of nutrients. Currently, sour pork is mainly manufactured through traditional natural fermentation (Cocolin *et al*., 2009; Charmpi *et al*., 2020). However, the quality of natural sour pork is still difficult to standardise because of various microorganisms and unstable fermentation processes that can lead to potential food safety issues (Charmpi *et al*., 2020). Therefore, the commercial production of sour pork results in considerable work needing to be done, in which obtaining access to available probiotic resources is the most important step. *Lactobacillus* is commonly used in the production of pork products (Luxananil *et al*., 2009). In the present work, we

obtained wild *L. plantarum* strain that could be domesticated into an industrial microorganism. The cholesterol in the sour pork produced *via* the obtained strain was decreased. Although a slow process, the obtained strain could be used at low temperature fermentation (10°C), and had a relatively wide ranged adaptability in temperature. The stability of the obtained strain was also investigated in a similar gastrointestinal internal environment (pH ranging from approximate 1.8 - 5.4 in gastric juice and 7.2 - 8.4 in intestinal juice) and in an extremely alkaline environment (pH ranging from 9.6 - 10.8). The obtained strain was stable in the gastrointestinal tract environment, thus suggesting a superiority in consumption.

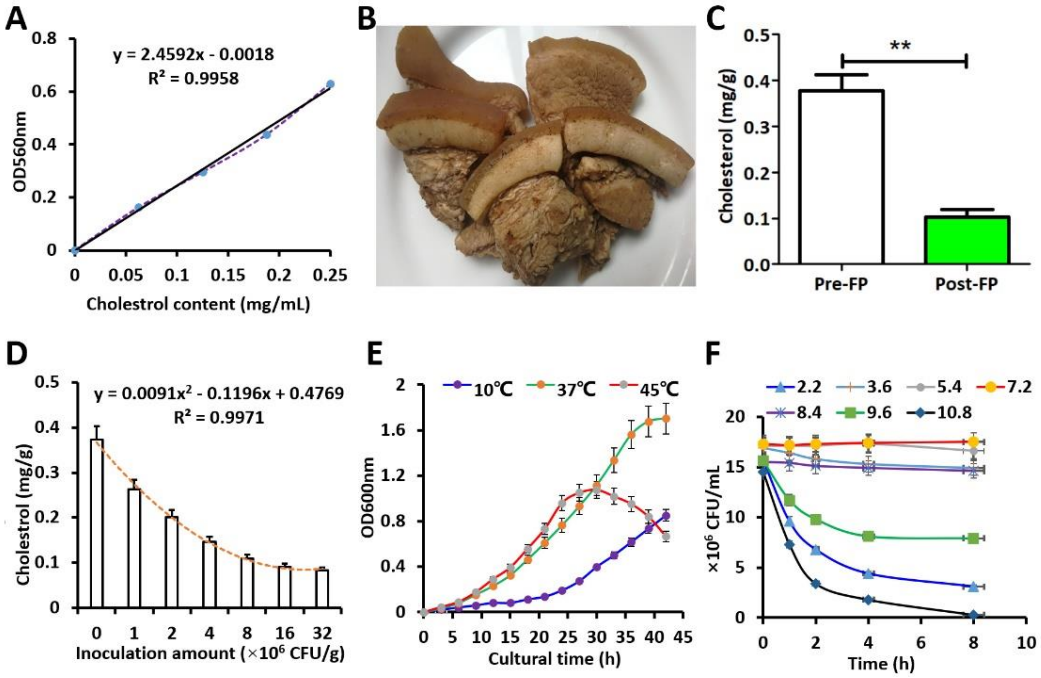

Figure 2. DC2 strain had the capacity to degrade cholesterol, and relatively wide-range adaptability in temperature and pH for producing sour pork. **(A)** Standard curve to detect cholesterol content; **(B)** fermented sour pork; **(C)** cholesterol content in pre- and post-fermentation sour pork; **(D)** effects of DC2 strain inoculation amount on degradation of pork cholesterol; **(E)** proliferation curve of DC2 strain under different temperature conditions; and **(F)** resistance of DC2 strains to different pH buffer solutions. $^{**}p < 0.01$ was considered to be statistically significant.

Dietary fermentation pork was beneficial for lowering blood lipids in mice

Cooked pre- and post-fermentation pork were used to feed the mice. The body weight in the post-FM group was significantly lower than that in the pre-FM group (Figure 3A), suggesting that the increasing trend of mice weight was slowed down when the mice was fed with sour pork. The serum levels of lipopolysaccharide-binding protein (LBP), total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides (TG) in mice serum were significantly downregulated compared to those in the pre-FM group (Figures 3B, 3C, 3E, and 3F). However, there were no significant differences in high-density lipoprotein levels between the pre- and post-FM groups (Figure 3D).

As an essential nutrient, cholesterol exists widely in various tissues and cells of the human body, and plays important physiological roles. However, excessive cholesterol accumulation in the body induces a variety of health problems. The daily cholesterol intake of residents in China has significantly increased from 165.8 mg/day 30 years ago to over 300 mg/day in recent years, and pork is the top of the three cholesterol sources (Su *et al*., 2015). A high cholesterol diet is the key risk factor for chronic metabolic disorders and cardiovascular diseases, which are partially accomplished through the activation of inflammatory pathways dependent on the regulation of LDL (Berger *et al*., 2015; Vinue *et al*., 2018). In the present work, we also found that mice fed with sour pork had lower levels of serum lipids, which may imply that dietary sour pork is a healthier lifestyle. However, the precise mechanism requires further studies.

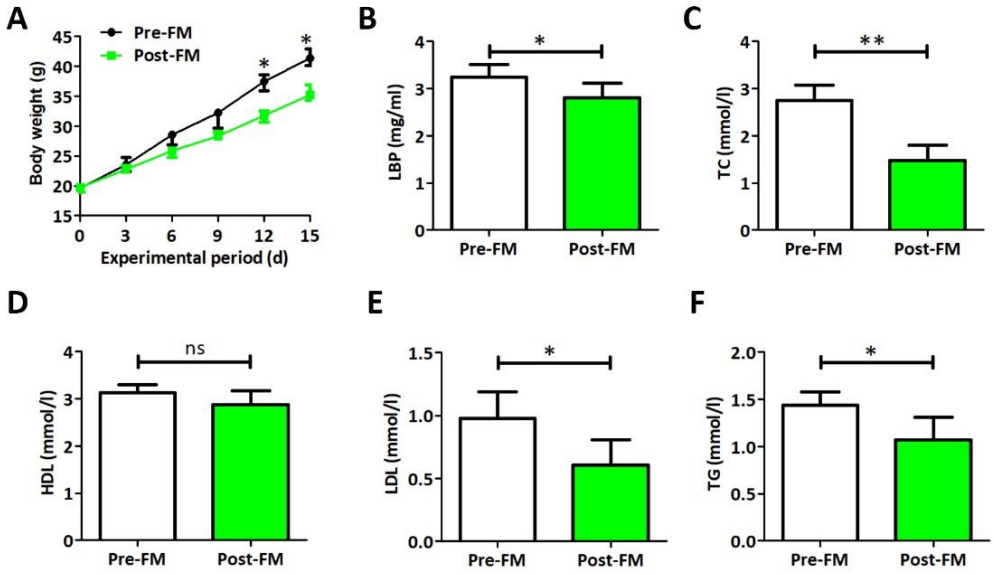

Figure 3. Body weight and blood lipid levels were downregulated in mice fed fermented sour pork, including LBP, TC, LDL, and TG (but not HDL). **(A)** Body weight; **(B)** serum lipopolysaccharide-binding protein (LPB); **(C)** serum total cholesterol (TC); **(D)** high density lipoprotein (HDL); **(E)** low density lipoprotein (LDL); and **(F)** triglycerides (TG). $^{**}p < 0.01$ and $^{*}p < 0.05$ were considered to be statistically significant. ns: $p > 0.05$ was considered to be nonsignificant.

Conclusion

In the present work, *Lactobacillus plantarum* (DC2 strain) with good capacity for cholesterol degradation was obtained (CCTCC NO: M 2019121) from traditional naturally fermented foods, and it could be used to produce sour pork through low temperature fermentation technology. Dietary sour pork is beneficial for deceasing blood lipids, which is important for people with impaired metabolisms or high cholesterol intake syndrome.

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References

Bales, C. W., Moreno, K. L., Guyton, J. R., Yunker, P. A., McGee, M. K., Currie, K. L., … and Drezner, M. K. 1998. Comparison of proximate composition and fatty acid and cholesterol content of lean and typical commercial pork. Journal of the American Dietetic Association 98: 1328-1330.

Berger, S., Raman, G., Vishwanathan, R., Jacques, P. F. and Johnson, E. J. 2015. Dietary cholesterol and cardiovascular disease: A systematic review and meta-analysis. American Journal of Clinical Nutrition 102: 276-294.

Charmpi, C., Van der Veken, D., Van Reckem, E., De Vuyst, L. and Leroy, F. 2020. Raw meat quality and salt levels affect the bacterial species diversity and community dynamics during the fermentation of pork mince. Food Microbiology 89: 103434.

Chinese Society of Nutrition (CSN). 2022. Dietary guidelines for Chinese residents. China: People's Medical Publishing House Co., Ltd.

Cocolin, L., Dolci, P., Rantsiou, K., Urso, R., Cantoni, C. and Comi, G. 2009. Lactic acid bacteria ecology of three traditional fermented sausages produced in the north of Italy as determined by molecular methods. Meat Science 82: 125-132.

Food and Drug Administration of China (FDAC). 2016. GB 5009.128-2016 - National Food Safety Standard - Determination of cholesterol in food.

Gu, X., Yang, L., Chi, F., Luo, Z., Ma, C. and Qiang, B. 2019. Nutritional composition analysis and evaluation of Tibetan swine pork. Journal of Nutrition 41: 513-515.

Guo, C., Su, C., Wang, Z., Du, W., Wang, H. and Zhang, B. 2018. Dietary protein intake of adults aged 18-64 in 15 provinces of China in 2015. Journal of Nutrition 40: 18-23.

Gusils, C., Chaia, A. P., Oliver, G. and Gonzalez, S. 2004. Microtechnique for identification of lactic acid bacteria. Methods in Molecular Biology 268: 453-458.

Herforth, A., Arimond, M., Alvarez-Sanchez, C., Coates, J., Christianson, K. and Muehlhoff, E. 2019. A global review of food-based dietary guidelines. Advances in Nutrition 10: 590-605.

Ling, D. and Dong, X. 1998. Classification and identification of lactic acid bacteria and experimental methods. Chinese Journal of Microbiology 1: 23.

Lu, Y. and Xiao, H. 2020. Chinese residents' meat consumption characteristics and trend judgment - Based on the double-log linear expenditure model and LA/AIDS model. Journal of China Agricultural University 25: 180-190.

Luxananil, P., Promchai, R., Wanasen, S., Kamdee, S., Thepkasikul, P., Plengvidhya, V., … and Valyasevi, R. 2009. Monitoring *Lactobacillus plantarum* BCC 9546 starter culture during fermentation of Nham, a traditional Thai pork sausage. International Journal of Food Microbiology 129: 312-315.

Song, S., Xia, T., Zhu, C., Xue, J., Fu, Q., Hua, C., … and Li, C. 2020. Effects of casein, chicken, and pork proteins on the regulation of body fat and blood inflammatory factors and metabolite patterns are largely dependent on the protein level and less attributable to the protein source. Journal of Agricultural and Food Chemistry 68: 9398-9407.

Stavropoulou, D. A., Filippou, P., De Smet, S., De Vuyst, L. and Leroy, F. 2018. Effect of temperature and pH on the community dynamics of coagulase-negative staphylococci during spontaneous meat fermentation in a model system. Food Microbiology 76: 180-188.

Su, C., Jia, X., Wang, Z., Wang, H. and Zhang, B. 2015. Trends in dietary cholesterol intake among Chinese adults: A longitudinal study from the China Health and Nutrition Survey, 1991-2011. BMJ Open 5: e007532.

Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512-526.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6 - Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Vinue, A., Herrero-Cervera, A. and Gonzalez-Navarro, H. 2018. Understanding the impact of dietary cholesterol on chronic metabolic diseases through studies in rodent models. Nutrients 10: 1-21.

Zhu, Z. and Ma, Y. 2020. Analysis of pork situation in 2019 and outlook for 2020. Pig Farming Today 1: 27-31.