Isolation, Identification, and Lead Adsorption of Lead-Resistant Strains from Healthy Newborn Feces: A Postprint

Authors: Shen Wei, Yu Qinfei, Wang Yuhao, Zhang Yiduo, Meng Xiaojing, Fan Hongying

Date: 2017-12-21T00:00:00+00:00

Abstract

Objective To isolate and identify lactic acid bacteria with heavy metal lead resistance and lead adsorption capacity, providing novel bacterial sources for functional foods with lead-excreting properties. Methods Using the lactic acid bacteria selective medium MRS, lead-resistant bacteria were isolated from 30 samples of healthy, full-term newborn feces. Through morphological characteristics, 16S rRNA sequencing, and phylogenetic tree construction; physiological and biochemical tests, antimicrobial susceptibility testing, and acid and bile salt tolerance tests were conducted according to Bergey' s Manual of Systematic Bacteriology; furthermore, the lead ion adsorption capacity of the isolated strains was detected by ICP-OES method. Results Three strains of Lactobacillus casei capable of tolerating 500 mg/L lead ion concentration were isolated, all sensitive to penicillin and ceftriaxone. Acid and bile salt tolerance tests showed that after 3 h cultivation in pH 2.0 artificial gastric juice, bacterial counts remained at the same order of magnitude, and survival rate reached 62.5% in 0.3% bile salt environment for 8 h. Adsorption experiments indicated that the adsorption rate for low-concentration (1 mg/L) lead ions was as high as 90.4%, and for high-concentration (50 mg/L) lead ions could reach 86.27%. Conclusion Three strains of Lactobacillus casei with lead resistance, lead adsorption capacity, and acid and bile salt tolerance were isolated from newborn feces, opening new avenues and solutions for alleviating lead poisoning effects through probiotic dietary strategies.

Full Text

Preamble

Journal of Southern Medical University, 2016, 36(12): 1602-1608
doi: 10.3969/j.issn.1673-4254.2016.12.03
ChinaXiv Cooperative Journal

Authors: SHEN Wei[1,2], YU Qinfei[2], WANG Yuhao[2], ZHANG Yiduo[2], MENG Xiaojing[2], FAN Hongying[2]

Affiliations:
[1]Department of Neonatology, Nanfang Hospital, Southern Medical University
[2]School of Public Health//Guangdong Provincial Key Laboratory of Tropical Disease Research, Southern Medical University, Guangzhou 510515, China

---

Abstract

Objective: To isolate and identify lead-resistant lactic acid bacteria with lead adsorption capacity from healthy newborn feces, providing novel bacterial strains for developing lead-detoxifying functional foods. **Methods:** Using Lactobacillus-selective MRS medium, lead-tolerant bacteria were isolated from 30 fecal samples of healthy, full-term neonates. Strains were characterized through morphological analysis, 16S rRNA sequencing, and phylogenetic tree construction. Physiological and biochemical properties were assessed following *Bergey' s Manual of Systematic Bacteriology*, complemented by antimicrobial susceptibility testing and acid/bile salt tolerance assays. Lead ion adsorption capacity was quantified by inductively coupled plasma-optical emission spectroscopy (ICP-OES). **Results:** Three *Lactobacillus casei* strains tolerating 500 mg/L lead ions were isolated, all showing susceptibility to penicillin and ceftriaxone. Acid and bile salt tolerance tests demonstrated that bacterial counts remained stable after 3 h in pH 2.0 artificial gastric juice, with survival rates reaching 62.5% following 8 h exposure to 0.3% bile salts. Adsorption experiments revealed a 90.4% removal rate for low-concentration (1 mg/L) lead ions and 86.27% for high-concentration (50 mg/L) lead ions. **Conclusion:** Three *Lactobacillus casei* strains with lead resistance, adsorption capacity, and tolerance to acid and bile salts were successfully isolated from newborn feces, offering a new approach and solution for alleviating lead poisoning through probiotic dietary strategies.

Keywords: lead; heavy metals; 16S rRNA; newborn; *Lactobacillus casei*; feces; adsorption

---

Introduction

According to World Health Organization statistics, 0.6% of the global disease burden is attributable to lead exposure, with approximately 600,000 new cases of childhood intellectual disability arising annually from lead exposure in children. Maternal lead exposure during pregnancy can also cross the placental barrier and blood-brain barrier, damaging fetal brain tissue and causing long-term sequelae. Traditional treatment of lead poisoning primarily relies on metal chelating agents that promote lead excretion, but their nephrotoxicity and interference with essential trace element absorption make them unsuitable for vulnerable populations such as pregnant women and children. Consequently, developing prevention and treatment strategies for lead accumulation poisoning in sensitive groups has become a major research focus.

Extensive scientific research has demonstrated that lactic acid bacteria, which are ubiquitous in the human gut, exhibit excellent safety profiles for dietary applications and have proven efficacy in preventing lactose intolerance, necrotizing enterocolitis in very low birth weight infants, antagonizing pathogenic bacteria, lowering cholesterol, and exerting anti-tumor and immunomodulatory effects. Drawing from experience in bioremediation of heavy metal environmental pollution, researchers have discovered that certain specific strains of lactic acid bacteria can effectively adsorb heavy metal ions in vitro, leading to the proposition of probiotics as a strategy for mitigating heavy metal toxicity. This provides a novel approach for exploring interventions against lead poisoning in special populations.

Halttunen et al. evaluated lead adsorption by classic strains including *Lactobacillus rhamnosus* GG (LGG), bifidobacteria, and *Lactobacillus casei*, demonstrating that LGG exhibits good lead-binding performance. However, implementing dietary strategies for preventing heavy metal lead poisoning requires identifying more microorganisms with Generally Recognized As Safe (GRAS) status that can tolerate heavy metals. Bhakta et al. isolated numerous lead-resistant lactic acid bacteria, but all were derived from heavy metal-contaminated sludge samples. Chen Wei et al. (2012) confirmed in animal studies that *Lactobacillus plantarum* CCFM8661, which has high lead affinity, could reduce blood lead and organ lead accumulation, but this strain originated from traditional Chinese fermented foods. Bisanz et al. conducted a study on pregnant women and children in Tanzania, a population at risk for heavy metal poisoning, administering yogurt fermented with *L. rhamnosus* GR-1 to the experimental group. The results showed lower blood mercury and arsenic levels compared to controls, providing the first evidence of heavy metal detoxification efficacy by probiotics in humans, though lead levels were not significantly reduced. Such studies remain scarce, and probiotic applications in lead-sensitive populations like pregnant women and children require additional safety considerations.

Therefore, this study selected healthy newborn feces as the source for isolating highly lead-tolerant lactic acid bacteria, ensuring relative safety of origin. Using traditional methods and PCR, we identified three highly lead-resistant *Lactobacillus casei* strains, evaluated their probiotic properties, and compared their lead adsorption performance with the classic strain LGG and the standard *L. casei* strain. Additionally, other experiments from our research group suggest these strains also exhibit high tolerance to multiple heavy metals including mercury, cadmium, manganese, and chromium—a finding not previously reported domestically or internationally. The strains isolated in this study provide high-quality bacterial sources for implementing dietary strategies to prevent heavy metal lead poisoning.

Materials and Methods

1.1 Sample Sources and Standard Strains

Thirty fecal specimens were collected from healthy newborns (age 28 days) in the postpartum unit of Nanfang Hospital, Southern Medical University. All infants were exclusively or predominantly breastfed and had not received antibiotics or probiotic preparations. Sample details are provided in Table 1 . The standard strain *Lactobacillus casei* subsp. *rhamnosus* ATCC7469 was purchased from the Guangdong Microbial Culture Collection Center.

1.2 Reagents

MRS solid medium, pepsin, and oxgall were obtained from Guangdong Huankai Microbial Technology. DL2000 DNA Marker, TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0, and TIANamp Bacteria DNA Kit were from TaKaRa. The novel microbial micro-biochemical identification tube series was from Guangdong Huankai Microbial Technology. GBW08619 lead single-element solution standard was used for preparing lead stock solution: 91.58 mg Pb(CH COO) was dissolved in 100 mL sterile distilled water to prepare a 500 mg/L stock solution, stored at 4°C protected from light. Artificial gastric juice was prepared by taking 16.4 mL of 0.1 g/L HCl, adjusting pH to 2.0, adding pepsin (0.01 g/L), dissolving thoroughly, sterilizing with 0.22 m membrane filtration, and storing at 4°C.

1.3 Instruments

Key equipment included: Whitley DG-250 anaerobic incubator, BIOER PCR thermal cycler, DYY-6C electrophoresis system, Tanon-1600 gel imaging system, SHINVA horizontal pressure steam sterilizer, Xinrui WGZ-2 turbidimeter, and Perkin Elmer Optima 7000DV ICP-OES.

1.2.1 Strain Isolation and Screening

Fresh fecal samples (30 total) were processed by adding sterile PBS at a 1:10 (g/mL) solid-liquid ratio, followed by serial dilution to 10 and 10 (60 tubes total). Cultivation began on MRS solid medium containing 50 mg/L initial lead ion concentration (lead acetate). After 48 h incubation at 37°C in an anaerobic chamber, distinct colony morphologies were identified. Different single colonies were randomly selected and inoculated into MRS medium containing 100 mg/L lead ions for 48 h. Surviving colonies were sequentially transferred to MRS media with 150 mg/L and 200 mg/L lead ions using the same method, followed by multiple rounds of isolation and purification. Colonies tolerating 200 mg/L lead ions were Gram-stained after smear preparation and stored in 20% glycerol at -80°C.

1.2.2 Maximum Metal Tolerance Concentration (MTC) Assay

Lead-resistant strains isolated in section 1.2.1 and ATCC7469 were inoculated onto MRS plates without heavy metal ions and incubated at 37°C for 48 h. Single colonies were suspended in 1 mL sterile water, and one loopful was streaked onto MRS plates containing 50, 100, 150, 200, 300, 400, and 500 mg/L Pb^2 . Growth was observed after 48 h, and the maximum metal tolerance concentration for each strain was recorded.

1.2.3 PCR Identification of Isolated Strains

1.2.3.1 Genomic DNA Extraction: Based on MTC results, strains tolerating 500 mg/L Pb^2 that were Gram-positive rods and catalase-negative were cultured for 24–48 h. Total genomic DNA was extracted using the TIANamp Bacteria DNA Kit.

1.2.3.2 PCR Amplification and 16S rDNA Identification: Universal primers for lactic acid bacteria 16S rDNA were used for PCR amplification. Forward primer 27f: 5' -AGAGTTTGATCCTGGCTCAG-3' ; reverse primer 1492r: 5' -GGTTACCTTGTTACGACTT-3' (synthesized by Shanghai Sangon Biotech). The 20 L reaction mixture contained 200 ng template, 10 L TaKaRa Premix Taq™, 0.5 mmol/L each primer, and ddH O to volume. Negative control used ddH O as template. PCR conditions: 94°C for 5 min; 30 cycles of 94°C for 60 s, 60°C for 60 s, 72°C for 90 s; final extension at 72°C for 10 min; hold at 4°C. PCR products were electrophoresed and sequenced by Shanghai Sangon Biotech. Results were analyzed by BLAST for 16S rRNA comparison, and a phylogenetic tree was constructed using MEGA7.0.

1.2.4 Probiotic Characterization

1.2.4.1 Physiological and Biochemical Tests: Physiological and biochemical characteristics of screened strains were identified according to *Bergey' s Manual of Systematic Bacteriology*, with ATCC7469 as control.

1.2.4.2 Drug Sensitivity Test: Agar diffusion disk method was used to determine sensitivity to basic antibiotics (penicillin, ceftriaxone, gentamicin) based on inhibition zone diameter.

1.2.4.3 Acid and Bile Salt Tolerance Tests: For acid tolerance, artificial gastric juice was prepared at pH 2.0, 3.0, and 4.0. Strains cultured to late logarithmic phase were washed with PBS and adjusted to 10^{1} cfu/mL. One milliliter of bacterial suspension was inoculated into 9 mL artificial gastric juice of different pH values and incubated anaerobically for 1-3 h. Viable counts before and after incubation were determined by pour plate method. For bile salt tolerance (0.3% bile salt concentration), acid-tolerant strains were placed in pH 3.0 phosphate buffer for 2 h anaerobic incubation at 37°C. Then 1 mL was transferred to 9 mL of 0.3% bile salt solution and incubated anaerobically at 37°C for 8 h. Viable counts before and after incubation were determined by pour plate method.

1.2.5 Adsorption Experiments

1.2.5.1 Solution Preparation: Lead ion solutions of 1, 10, 50, and 100 mg/L were prepared and pH adjusted to 6.0.

1.2.5.2 Bacterial Enrichment: Strains tolerating 500 mg/L Pb^{2} that were Gram-positive rods and catalase-negative, along with ATCC7469, were cultured for 24 h. Cultures were diluted to OD=1 and enumerated by plate counting.

1.2.5.3 Bacterial Pretreatment: One milliliter of bacterial suspension was centrifuged (8000 r/min, 10 min) to collect cells. Cells were resuspended in lead solutions to a final concentration of 2 g/L and incubated at 37°C on a shaker (120 r/min) for 2 h. For heat-killed bacteria, equivalent cell amounts were boiled for 1 h before addition to lead solutions.

1.2.5.4 Sample Detection: After incubation, samples were centrifuged (8000 r/min, 10 min). Supernatants were collected and analyzed for lead ion concentration by ICP-OES. Analysis conditions: wavelength 220.353 nm, plasma flow 15 L/min, nebulizer flow 0.8 L/min, auxiliary flow 0.2 L/min, RF power 1100 W, axial plasma viewing, sample flow rate 1.5 mL/min.

1.2.5.5 Calculation of Adsorption Rate and Capacity:

Adsorption capacity (mg/g):

$$Q_e = \frac{(C_0 - C_e)}{m} \times V$$

Adsorption rate (%):

$$Q_r = \frac{(C_0 - C_e)}{C_0} \times 100\%$$

Where Q_e represents adsorption capacity (mg/g), Q_r represents adsorption rate (%), C_0 is initial lead ion concentration (mg/L), C_e is final lead ion concentration after adsorption (mg/L), m is bacterial mass (g), and V is total solution volume (L).

1.3 Data Processing

Data were analyzed using GraphPad Prism 7.02. Quantitative data are expressed as mean ± standard deviation. Inter-group comparisons were performed using t-tests, with $P<0.05$ considered statistically significant. All experiments were repeated three times.

Results

2.1 Screening and Purification of Lead-Resistant Strains

All 30 fecal samples showed abundant colony growth on MRS+Pb^{2} medium at the initial concentration of 50 mg/L. Ten single colonies with Pb^{2} MTC of 200 mg/L were observed. After increasing Pb^{2} concentration, three strains with Pb^{2} MTC reaching 500 mg/L and good growth were obtained (all from exclusively breastfed infants), designated SYF-B, SYF-E, and SYF-I. The standard strain ATCC7469 showed growth inhibition at 300 mg/L Pb^{2} .

2.2 Morphological Observations

Preliminary identification of the three screened strains revealed colonies that were milky white, moist, circular with raised centers and regular edges, approximately 1–2 mm in diameter. Microscopic examination showed Gram-positive rods without spores, consistent with ATCC7469 staining characteristics (Figure 1 [Figure 1: see original paper]).

2.3 Identification of Lead-Resistant Strains

Genomic DNA was extracted from the three lead-resistant strains for PCR amplification, yielding clear bands at the target position (Figure 2 [Figure 2: see original paper]). The 16S rRNA sequences were determined by Shanghai Sangon Biotech and analyzed by BLAST. The results showed that all three strains were highly homologous to 16S rRNA sequences of various *Lactobacillus casei* strains, with identity rates of 100%, 99%, and 100%, respectively (Table 2). A phylogenetic tree constructed using the Neighbor-joining method in MEGA7.0 software was consistent with BLAST analysis, clustering the isolated strains with *L. casei* strains (Figure 3 [Figure 3: see original paper]), indicating their closest phylogenetic relationship. Based on colony characteristics, bacterial morphology, and 16S rRNA sequence analysis, all three strains were identified as *Lactobacillus casei*.

2.4 Physiological and Biochemical Tests

According to *Bergey' s Manual of Systematic Bacteriology*, physiological and biochemical tests showed that all three strains exhibited biological characteristics consistent with *Lactobacillus casei* non-*rhamnosus* subspecies (Table 3).

2.5 Drug Sensitivity Test Results

All three lead-resistant strains were resistant to gentamicin but sensitive to penicillin and ceftriaxone, indicating that basic antibiotics such as penicillin can effectively inhibit their growth (Figure 4 [Figure 4: see original paper]).

2.6 Artificial Gastric Juice Test

Human gastric pH is typically around 2.0, gradually decreasing to approximately pH 5.0 after meals, with gastric emptying time generally 3–4 h. Therefore, foodborne anti-lead probiotics should possess good acid tolerance. Our results showed that the three lead-resistant strains tolerated acidic environments well (Table 4). At pH 3.0 and 4.0, bacteria continued to grow; at pH 2.0, counts remained at 10^1 cfu/mL after 3 h, demonstrating tolerance to gastric acid. Bile salt tolerance followed the pattern SYF-I > SYF-E > SYF-B, with 8 h survival rates reaching 62.5% (Table 5).

2.7 Adsorption Experiments

Various initial lead ion concentrations were tested. At low concentration (1 mg/L), all strains showed high adsorption rates of approximately 83.2–96.7%, with minimal difference between viable and heat-killed bacteria. As initial lead ion concentration increased, adsorption approached saturation, with rates gradually decreasing while adsorption capacity increased, consistent with literature reports. Under conditions of pH 6.0, bacterial concentration 2 g/L, and lead ion concentration 50 mg/L, all three isolated strains achieved adsorption rates above 60%. Notably, SYF-I heat-killed bacteria showed much higher adsorption capacity than viable cells, while SYF-E showed moderate adsorption capacity for both viable and heat-killed cells (Figure 5 [Figure 5: see original paper]). LGG heat-killed bacteria exhibited very high adsorption rates (91.17%), but its viable cell adsorption capacity was inferior to SYF-B (86.27%, Figure 6 [Figure 6: see original paper]).

Discussion

Identifying microorganisms with GRAS status that can tolerate heavy metals is the first step in dietary lead poisoning prevention. While environmental remediation studies have demonstrated exceptional microbial adsorption of heavy metals, most specimens originate from contaminated soil, water, and plants, raising safety concerns if used as food additives for heavy metal removal. Between 7–10 days after birth, bifidobacteria and lactobacilli in the infant gut reach a proliferation peak, comprising over 90% of the total gut microbiota. At this stage, pathogenic bacteria are scarce and strains are relatively uniform, with few drug-resistant bacteria and minimal environmental adaptation-induced mutations, facilitating isolation of desired probiotics. These characteristics prompted our use of newborn fecal specimens for screening food-safe probiotic strains.

The three human-derived *Lactobacillus* strains SYF-B, SYF-E, and SYF-I were identified as *L. casei* through traditional and PCR methods. *Lactobacillus casei* is a common probiotic with multiple functions including cholesterol reduction, bacteriocin production, cell division promotion, and antibody-mediated immunity. Our strains, isolated from healthy newborn feces, tolerated 500 mg/L lead ions—well exceeding the 100 g/L blood lead level requiring social intervention in children—thus fully meeting requirements for dietary prevention research. The strains demonstrated the following probiotic properties: (1) They originated from the newborn gut and adapted well to simulated human digestive conditions, maintaining counts above 10^1 cfu/mL after 3 h at pH 2.0 and achieving 62.5% survival after 8 h in 0.3% bile salts, superior to other reports on human-derived lactobacilli; (2) Drug sensitivity tests showed basic antibiotics like penicillin effectively inhibit all three strains, ensuring controllability for applications in pregnant women and children; (3) They possess excellent adsorption capacity. Compared with standard strain ATCC7469 and classic strain LGG, SYF-B showed outstanding adsorption capacity, with viable cell performance even surpassing LGG at 50 mg/L lead concentration. In Halttunen et al.' s study, the classic *L. casei* Shirota strain also showed lead adsorption capacity, but its viable cell adsorption rate was lower than SYF-B when calculated from their reported values. Maintaining probiotic viability will facilitate multiple functional benefits. Additionally, heat-killed bacteria adsorption capacity suggests these strains could serve as lead detoxifiers for immunocompromised populations, such as premature infants requiring multiple blood transfusions who may experience lead accumulation. SYF-I' s characteristics align with this application.

Therefore, the three *Lactobacillus casei* strains SYF-B, SYF-E, and SYF-I demonstrate potential value as heavy metal detoxifiers for pregnant women and children. Future studies should investigate their intestinal adhesion properties, beneficial metabolites, and in vivo lead adsorption capacity to ultimately provide new dietary strategies for alleviating lead poisoning effects in these sensitive populations.

References

[1] Chen Xinxin, Teng Honghong, Wang Fengzhi, et al. Study on blood lead levels and related influencing factors in children aged 0-6 years in Beijing [J]. *Chinese Journal of Epidemiology*, 2003, 24(10): 868-71.

[2] Ozkiraz Servet, Gokmen Zeynel, Ecevit Ayse, et al. Vestibular evoked myogenic potentials in term newborn infants with severe hyperbilirubinemia [J]. *Pediatric International*, 2012, 54(5): 646-50.

[3] Porru S, Alessio L. The use of chelating agents in occupational lead poisoning

[J]. *Occupational Medicine*, 1996, 46(1): 41-8.

[4] Aposhian V, Maiorino M, Gonzalez-Ramirez D, et al. Mobilization of heavy metals by newer, therapeutically useful chelating agents [J]. *Toxicology*, 1995, 97(1/3): 23-38.

[5] Lidsky I, Schneider S. Lead neurotoxicity in children: basic mechanisms and clinical correlates [J]. *Brain*, 2003, 126(Pt 1): 5-19.

[6] Thomas DW, Greer FR, Nutr C. Clinical Report—Probiotics and prebiotics in pediatrics [J]. *Pediatrics*, 2010, 126(6): 1217-31.

[7] Frye E, Rose Shannon, Slattery John, et al. Gastrointestinal dysfunction in autism spectrum disorder: role of mitochondria and the enteric microbiome [J]. *Microbial Ecology in Health and Disease*, 2015, 26(7): 27458.

[8] Kirtzalidou E, Pramateftaki P, Kotsou M, et al. Screening for lactobacilli with probiotic properties in the infant gut microbiota [J]. *Anaerobe*, 2011, 17(6): 440-3.

[9] Chinese Pharmacopoeia Commission. *Pharmacopoeia of the People' s Republic of China* [M]. Beijing: Chemical Industry Press, 2010.

[10] Jankovic I, Sybesma W, Phothirath P, et al. Application of probiotics in food products—challenges and new approaches [J]. *Current Opinion in Biotechnology*, 2010, 21(2): 175-81.

[11] Rijkers GT, Bengmark S, Enck P, et al. Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research [J]. *Journal of Nutrition*, 2010, 140(3): 671S-6S.

[12] Topcu A, Bulat T. Removal of cadmium and lead from aqueous solution by *Enterococcus faecium* strains [J]. *Journal of Food Science*, 2010, 75(1): T13-7.

[13] Bhakta N, Ohnishi K, Munekage Y, et al. Characterization of lactic acid bacteria-based probiotics as potential heavy metal sorbents [J]. *Journal of Applied Microbiology*, 2012, 112(6): 1193-206.

[14] Wang Jianlong, Chen Can. Research progress on removal of heavy metal ions by biosorption [J]. *Acta Scientiae Circumstantiae*, 2010, 30(4): 673-701.

[15] Urban PL, Kuthan RT. Application of probiotics in the xenobiotic detoxification therapy [J]. *Nukleonika*, 2004, 49(1): S43-5.

[16] Taibi Amel, Comelli M. Practical approaches to probiotics use [J]. *Applied Physiology, Nutrition, and Metabolism*, 2014, 39(8): 980-6.

[17] Neltner G, Alger M, O' reilly T, et al. Conflicts of interest approvals of additives to food determined to be generally recognized as safe: out of balance [J]. *JAMA Internal Medicine*, 2013, 173(22): 2032-6.

[18] De Lacerda JR, Da Silva TF, Vollú RE, et al. Generally recognized as safe (GRAS) *Lactococcus lactis* strains associated with *Lippia sidoides* Cham. are

able to solubilize/mineralize phosphate [J]. *SpringerPlus*, 2016, 5(1): 828.

[19] Bisanz E, Enos K, Mwanga R, et al. Randomized open-label pilot study of the influence of probiotics and the gut microbiome on toxic metal levels in Tanzanian pregnant women and school children [J]. *mBio*, 2014, 5(5): e01514-80.

[20] Yu Jinghua. Isolation, screening, functional properties and application of intestinal probiotics from healthy humans [D]. Beijing: China Agricultural University, 2006.

[21] Blakemore RP, Blakemore NA, Bazylinski DA, et al. *Bergey' s Manual of Systematic Bacteriology* [M]. Baltimore MD: Williams and Wilkins, 1989.

[22] Yu Shangfu, Xu Min, Ding Xiuyun, et al. Optimization of Pb^{2} adsorption conditions by a lactic acid bacterium [J]. *China Brewing*, 2016, 35(2): 109-14.

[23] Halttunen T, Salminen S, Tahvonen R. Rapid removal of lead and cadmium from water by specific lactic acid bacteria [J]. *International Journal of Food Microbiology*, 2007, 114(1): 30-5.

[24] Ni Yihong. Screening of copper-tolerant lactic acid bacteria and study on their copper-binding properties [D]. Guangzhou: Jinan University, 2014.

[25] Hofmann AF. The enterohepatic circulation of bile acids in man [M]. Philadelphia: Saunders, 1977: 3-24.

[26] Mrvčić Jasna, Stanzer Damir, Solić Ema, et al. Interaction of lactic acid bacteria with metal ions: opportunities for improving food safety and quality [J]. *World Journal of Microbiology and Biotechnology*, 2012, 28(9): 2771-82.

[27] Amara A, Shibl A. Role of probiotics in health improvement, infection control and disease treatment and management [J]. *Saudi Pharmaceutical Journal*, 2015, 23(2): 107-14.

[28] Liu Ping, Guo Zhenhong, Dong Lina, et al. Characteristics of intestinal flora in healthy people of different ages by real-time fluorescent quantitative PCR [J]. *China Clinical Practical Medicine*, 2016, 7(1): 24-7.

[29] Ministry of Health of the People' s Republic of China. Principles for classification and management of childhood hyperleademia and lead poisoning [Z], 2006.

[30] Chen Xin, Pan Suhua, Hu Lili, et al. Determination and identification of acid and bile salt tolerance of human-derived *Lactobacillus* [J]. *Modern Preventive Medicine*, 2006, 33(3): 278-9.

[31] Atkinson M, Budge H. Review of the NICE guidance on neonatal jaundice [J]. *Archives of Disease in Childhood: Education and Practice Edition*, 2011, 96(4): 136-40.

[32] Tian Fengwei, Zhai Qixiao, Zhao Jianxin, et al. *Lactobacillus plantarum* CCFM8661 alleviates lead toxicity in mice [J]. *Biological Trace Element Research*, 2012, 150(1/3): 264-71.

Note: All figure and table citations ([FIGURE:N], [TABLE:N]) have been preserved exactly as in the original text. Mathematical formulas in section 1.2.5.5 remain unchanged.

Note: Figure translations are in progress. See original paper for figures.