Science of the Total Environment 958 (2025) 177997

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Consuming probiotics protects against cadmium exposure from rice consumption while promotes gut health: An assessment based on a mouse model

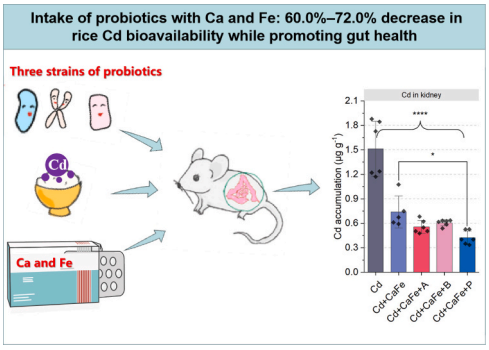
Jin-Feng Xi, Lei Zhou, Yao-Sheng Zhang, Xin-Ying Lin, Shan Chen, Rong-Yue Xue, Dongmei Zhou, Hong-Bo Li *

State Key Laboratory of Pollution Control and Resource Reuse, Jiangsu Key Laboratory of Vehicle Emissions Control, School of Environment, Nanjing University, Nanjing 210023, China

HIGHLIGHTS

- Intake of probiotics promoted growth of beneficial bacteria in the gut.
- Intake of probiotics promoted rice-Cd excretion via feces.
- Intake of probiotics caused up-regulation of intestinal Ca and Fe transporters.
- Intake of probiotics lower rice-Cd accumulation in mouse tissue insignificantly.
- Intake of probiotics alongside with Ca and Fe reduced rice-Cd accumulation in mouse kidneys by 60.0 %–72.0 %.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Fang Wang

Keywords:
Cd-contaminated rice
Probiotics
Gut microbiota
Mineral status
Mice

ABSTRACT

Cadmium (Cd) in rice constitutes a global health risk. It is crucial to develop strategies that not only reduce the bioavailability of Cd in rice but also confer additional health benefits. One potential approach involves the consumption of probiotics, which can bind Cd in the intestines and enhance gut health. The effects of consuming *Akkermansia muciniphila*, *Bifidobacterium pseudolongum*, and *Psychrobacter* sp. on the bioavailability of Cd in rice and gut health were evaluated using *in vivo* mouse bioassays and *in vitro* Cd immobilization assays. In mice fed Cd-contaminated rice without dietary calcium (Ca) and iron (Fe) supplementation (i.e., under conditions of mineral deficiency), the intake of these probiotics insignificantly reduced Cd accumulation in the kidneys and livers, although it did promote Cd excretion via feces. This outcome was primarily due to the competition for Ca and Fe between the probiotics and the host, which led to increased intestinal expression of Ca and Fe transporters under mineral-deficient conditions, thereby mitigating the probiotics' ability to reduce Cd bioavailability. Conversely, in mice fed Cd-contaminated rice with adequate dietary Ca and Fe (i.e., under conditions of mineral adequacy), probiotic intake significantly decreased Cd concentrations in the kidneys by 60.0 %–72.0 % compared to the control group exposed to Cd. Additionally, probiotic consumption fostered the growth of beneficial gut bacteria and strengthened intestinal tight junctions, reducing the inflammatory response in the

* Corresponding author.
E-mail address: hongboli@nju.edu.cn (H.-B. Li).

https://doi.org/10.1016/j.scitotenv.2024.177997
Received 8 October 2024; Received in revised form 17 November 2024; Accepted 5 December 2024
Available online 12 December 2024

intestines. These findings suggest that combining probiotics with sufficient Ca and Fe intake can effectively reduce dietary Cd exposure and enhance gut health.

1. Introduction

Cadmium (Cd) is a toxic metal that is ubiquitously present in the environment (Peana et al., 2023). Once ingested, Cd accumulates in various organs and exhibits a long biological half-life, ranging from 10 to 30 years (Järup and Åkesson, 2009). Exposure to Cd has been associated with increased risks of various cancers (Park et al., 2008), including breast (Gallagher et al., 2010), prostate (Julin et al., 2012), thyroid (Park et al., 2021), and kidney (Shi et al., 2021). Rice, which is cultivated in at least 114 countries, serves as a staple food for a significant portion of the global population (Sharif et al., 2014). However, Cd tends to accumulate in rice grains from contaminated soil. Several countries, including India (Sharma et al., 2018), Japan (Nogawa et al., 2004), Iran (Shakerian et al., 2012), and China (Liu et al., 2022), have reported instances of Cd contamination in rice. Approximately 5 % of the global rice supply exceeds the European Standard threshold of 0.2 μg g^{-1} (Shi et al., 2020). Consequently, rice consumption represents a significant pathway for Cd exposure to humans worldwide (Li et al., 2017).

In addition to controlling Cd concentration in rice grains, reducing the bioavailability of Cd in the intestine presents new opportunities to decrease human exposure to Cd. Cadmium bioavailability is defined as the proportion of ingested Cd that is absorbed across the intestinal barrier and enters the systemic circulation (Li et al., 2019). As a non-essential metal, Cd often utilizes the same transport pathways as essential minerals, entering enterocytes through iron (Fe) and calcium (Ca) transporters, such as the divalent metal transporter 1 (DMT1), responsible for transporting ferrous iron (Fe^{2+}) (Garrick et al., 2003; Park et al., 2002), and the apical Ca^{2+} influx channel (TRPV6) (de Barboza et al., 2015; Martinez-Finley et al., 2012). This similarity provides a significant opportunity to reduce rice Cd bioavailability by increasing dietary Ca and Fe intake (Li et al., 2022; Zhao et al., 2017), primarily through competition between these minerals and Cd for transport and the down-regulated expression of intestinal Ca and Fe transporters (Brasse-Lagnel et al., 2011; Kiela and Ghishan, 2018). Despite its effectiveness, this approach has limitations, including potential health risks such as cardiovascular diseases, constipation, diarrhea, colorectal tumorigenesis, and liver injury associated with excessive Ca and Fe intakes (Anderson and Klemmer, 2013; Li et al., 2018; Liu et al., 2023a, 2023b).

It is crucial to develop new strategies that can reduce the bioavailability of Cd in rice without introducing additional health risks, and potentially even confer health benefits. One promising approach is the consumption of probiotics, which have demonstrated a high capacity for binding potentially toxic elements, including Cd (Alizadeh et al., 2022). Probiotics, defined as live microorganisms that confer health benefits when consumed in adequate amounts (Hill et al., 2014), have shown various health advantages. For instance, *Akkermansia muciniphila* has been shown to reduce intestinal inflammation, strengthen intestinal tight junctions, and improve the gut microbiota by promoting the growth of beneficial bacteria (Zhang et al., 2022). Additionally, *A. muciniphila* has shown potential in mitigating colitis and improving conditions such as obesity and diabetes (Plovier et al., 2017; Wang et al., 2020a). Similar benefits have been observed with *Bifidobacterium* species (Badgeley et al., 2021; Kim et al., 2022). Given these advantages, probiotics have been incorporated into a wide range of products, including medical foods, beverages, ice cream, yogurt, bread, and infant formulas (Latif et al., 2024; Reque and Brandelli, 2021). The global probiotics market has rapidly expanded from an estimated US$ 47.6 billion in 2021 to US$ 73.1 billion in 2023 (Wiegers et al., 2023). Additionally, functional groups such as phosphate, hydroxyl, and amino groups on probiotics have been shown to bind Cd (Kurultak et al., 2022; Wang et al., 2020b). Probiotics can also bioaccumulate Cd within their cells through transmembrane transport (Mrvcic et al., 2012). For example, *Lactobacillus rhamnosus* GR-1 has immobilized Cd in vitro (Daisley et al., 2019), while *Lactobacillus fermentum* ME3 and *Bifidobacterium longum* 46 have removed Cd from water (Teem et al., 2008). Given the potential of probiotics to reduce Cd solubility in the gastrointestinal tract, along with their benefits in promoting beneficial gut bacteria and enhancing intestinal barrier function, it is hypothesized that probiotic consumption could reduce rice-Cd bioavailability and alleviate intestinal toxicity.

However, uncertainties remain regarding the effectiveness of probiotics in reducing Cd bioavailability, particularly concerning their impact on the bioavailability of Ca and Fe to the host. Probiotic strains require these essential metal nutrients for growth and function, which can lead to competition between the host and intestinal microflora (Huynh et al., 2022; Reeves and Chaney, 2008). Notably, under conditions of low mineral intake, intestinal microflora may significantly compete with the host for Ca and Fe, potentially leading to mineral deficiencies in the host (Nalepa et al., 2012). This competition may prompt the host to upregulate the expression of intestinal Ca and Fe transporters, thereby enhancing the efficiency of transcellular intestinal Cd transport. Ultimately, this process could negate the probiotics' ability to reduce Cd solubility in the intestinal lumen, thus obscuring the net effects on rice Cd bioavailability.

The aim of this study is to elucidate the ability of probiotics to reduce the bioavailability of Cd in rice and alleviate its intestinal toxicity under conditions of mineral deficiency and adequacy, using mouse bioassays. Drawing on previous research, we assessed three probiotic strains: *Akkermansia muciniphila* (ATCC BAA-835), *Bifidobacterium pseudolongum* (ATCC 15707), and *Psychrobacter* sp. (CICC 24001), which have demonstrated effectiveness in mitigating arsenic exposure (Zhang et al., 2023). The specific objectives are to: (1) examine the effects of these strains on gut microbiota, Cd excretion in feces, and the accumulation of Cd, Ca, and Fe in mouse tissues, as well as the intestinal expression of Ca and Fe transporters under conditions of Cd exposure from contaminated rice, both with and without dietary supplementation of Ca and Fe; and (2) explore the roles of these strains in reducing the risk of intestinal inflammation. Understanding the mechanisms by which probiotic supplementation reduces rice-Cd bioavailability is essential for efficiently leveraging the benefits of gut probiotics to decrease both Cd bioavailability and toxicity, thereby conferring health benefits.

2. Materials and methods

2.1. Rice

A Cd-contaminated white rice (TL rice) was collected from a copper mining impacted area located in Tongling city of Anhui province, while a Cd-free white rice (NJ rice) was purchased from the market in Nanjing city of Jiangsu province of China. Subsamples were digested according to USEPA Method 3050B (USEPA, 1996) prior to determination of Cd concentration using inductively coupled plasma mass spectrometry (ICP-MS, NexION300X, Perkin Elmer, USA) and Ca and Fe concentrations using inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300DV, PerkinElmer, US). The Cd, Ca, and Fe concentrations were 0.37 ± 0.007, 133 ± 29.3, and 4.89 ± 2.74 μg g^{-1} in the TL rice and 0.013 ± 0.0002, 105 ± 0.79, and 5.32 ± 0.65 μg g^{-1} in the NJ rice. The two samples were cooked (rice:water = 1:1.2, weight basis; boiling for 30 min), kneaded into rice balls (~2 cm diameter), and freeze-dried.

2.2. Probiotics

A. muciniphila (ATCC BAA-835), *B. pseudolongum* (ATCC 15707), and *Psychrobacter* sp. were purchased from China Center of Industrial Culture Collection. As anaerobic bacteria, *A. muciniphila* and *B. pseudolongum* were cultured in Fluid Thioglycollate (FT) medium at 37 °C for 40 h and 16 h without shaking to reach the logarithmic phase. In comparison, *Psychrobacter* sp. was cultured in Tryptic Soy Broth (TSB) medium at 28 °C for 10 h with shaking at 220 rpm. At the end of the culture, bacterial pellets were collected via centrifugation (10,000 rpm, 15 min) after measuring the OD_{600} value to get bacteria for supply to mice.

2.3. Mouse bioassays

Mice have been utilized to assess the bioavailability of Cd in rice, with the concentration of Cd in the kidneys serving as the endpoint (Hernandez-Cruz et al., 2022). To evaluate the impact of probiotics on rice-Cd bioavailability, sixty pathogen-free BALB/c female mice, four weeks old and approximately 15 g in body weight, were acquired from Nanjing Junke Bioengineering Co. Ltd., China. These mice were housed in polyethylene cages with dry wood shaving bedding under controlled conditions of 25 °C, 50 % humidity, and a 12/12-h light/dark cycle. All experimental procedures involving mice were conducted in compliance with China's guiding principles for the use of experimental animals. After a three-day acclimation period with Cd-free NJ rice, the mice were subjected to the following two bioassays.

(a) Intake of probiotics under mineral deficiency

For this assay, 5 mouse groups were included: (1) negative control group fed the Cd-free NJ rice; (2) Cd exposure control group fed the Cd-contaminated TL rice; (3–5) probiotics treated groups fed the TL rice and daily gavaged with *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp., respectively (Cd + A, Cd + B, and Cd + P). During the assay, mice were not supplied with any exogenous Ca or Fe to represent low mineral intake status.

(b) Intake of probiotics under mineral adequacy

To assess effects of probiotics on rice Cd bioavailability under mineral adequacy, the TL rice was amended with $CaCO_3$ at 500 μg g^{-1} Ca, while drinking water was added with $FeSO_4$ at 50 mg L^{-1} Fe. We selected $CaCO_3$ rather than other Ca supplements such as Ca citrate or Ca hydrogen phosphate to avoid the influences of organic ligands or phosphate on Cd bioavailability. Then, 5 mouse groups were set: (1) Cd exposure control group fed the TL rice without probiotics, Ca, or Fe ingestion; (2) Ca and Fe treated group fed $CaCO_3$-amended TL rice and receiving water containing Fe (Cd + CaFe); (3–5) probiotics and mineral co-treated groups fed $CaCO_3$-amended rice TL, receiving water containing Fe, and daily administered with *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. via gavage, respectively (Cd + CaFe+A, Cd + CaFe+B, and Cd + CaFe+P). To prevent oxidation of ferrous iron to ferric iron, we supplemented ferrous iron through water and replaced it with freshly prepared solution every two days.

For probiotic-treated groups, pre-cultured bacteria suspended in 100 μL sterile water [5×10^8 colony-forming units (CFU) per mouse] were orally administered to mice daily at 9:00 am via gavage. For each group, 6 mice housed individually in 6 separated cages were used as replicates based on previous studies (Jafarpour et al., 2017). All mice were sufficiently available with corresponding rice and drinking water over 14 d with body weight gain being recorded, while the total diet consumption was calculated as the differences in diet supplied and remaining. Mice were sacrificed via cervical dislocation to collect serum by clotting at room temperature for 1 h in coagulation-promoting tubes and centrifuging at 3000 rpm, 4 °C for 10 min. Mouse tissues including liver, kidney, duodenum, and ileum were collected. The luminal contents from the cecum were collected and stored at −80 °C prior to gut microbiota analyses, as the cecum acts as a large reservoir of symbiotic microorganisms. Feces samples were collected for Cd concentration and fractionation analyses.

2.4. Cd and Minerals in Mouse Tissue and Feces

Mouse liver, kidney, and feces samples were freeze-dried, digested according to USEPA Method 3050B (USEPA, 1996), and measured for Cd using ICP-MS and Ca and Fe using ICP-OES. To show Cd immobilization in the intestine by probiotics, a sequential extraction method was applied to fractionate Cd in mouse feces to 1 M $MgCl_2$ extractable, sodium acetate (pH = 5.0) extractable, 0.04 M $NH_2OH{\cdot}HCl$ extractable, organic matter bound, and residual fractions (Abollino et al., 2002; Tessier et al., 1980). Details are provided in the supporting information (SI).

2.5. Serum and Liver Biochemical Analyses

To assess the modulating effects of probiotics administration on the inflammation risk of rice Cd exposure, serum levels of interleukin 1β (IL-1β) were measured using a mouse ELISA Kit (CB10173-Mu; Shanghai Keaibo Bio-technology Co., Ltd., China). To assess changes in body Fe status, liver hepcidin was measured using a mouse ELISA Kit (CB10661-Mu; Shanghai Keaibo Bio-technology Co., Ltd., China). Hepcidin is a liver-derived peptide hormone that controls body Fe homeostasis (Collins et al., 2018). It can bind to ferroportin 1 (FPN1) that located on the basolateral membranes of duodenal enterocytes, causing FPN1 internalization and degradation. So, hepcidin is often induced by high body Fe content and reduced during Fe deficiency (Collins et al., 2018).

2.6. Gut Microbiota Analyses

The role of probiotics administration in modulating gut microbiota was assessed. Cecal content samples from mice of negative control, Cd control, Cd + A, Cd + B, and Cd + P ($n = 6$ for each treatment) were individually extracted using a Stool Genomic DNA kit (CoWin Biotech, Beijing, China). The integrity of the genomic DNA was detected by 1 % Agarose gel electrophoresis. Subsequently, the extracted DNA were amplified using PCR (GeneAmp9700, ABI) targeting the V3–V4 regions of 16S rRNA gene with PCR products being quantified using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, USA). The amplicons were paired-end sequenced using an Illumina Novaseq6000 platform (Genepioneer Biotechnologies, Shanghai, China). Raw sequence data were deposited in NCBI SRA under accession number PRJNA1138677. Details on sequence data processing are provided in the SI.

2.7. Quantitative RT-PCR

To assess intestinal Cd transport, mRNA expression of *Trpv6* and *Cabp-d_{9k}* that encoded apical Ca^{2+} influx transporter TRPV6 and intracellular Ca^{2+} buffering protein calbindin-D_9k (D9K) and mRNA expression of *Dmt1* that encoded apical Fe^{2+} influx transporter DMT1 were quantified in the duodenum of mice because duodenum is the major site of metal absorption (Walters et al., 2006; Zhang et al., 2016). To evaluate the modulating effects of probiotics administration on intestinal inflammation risk, the gene expression of intestinal tight junctions [*Zona-Occludins-1* (*Zo-1*), *Claudin 1*, and *Claudin 4*] and pro-inflammatory factors (*Il-1β*, *Il-6*, and *Tnf-α*) in the ileum of mice was also quantified. Details are shown in the SI with the primers of target mRNAs and the internal control mRNA (*β-actin*) being shown in Table S1. The mRNA expression data are expressed using the delta delta Ct method (Livak and Schmittgen, 2001).

2.8. Cd Immobilization by Probiotics in Vitro

To explain reduction in rice Cd bioavailability in mice with probiotics administration, Cd immobilization by probiotics was assessed in vitro. Briefly, pre-cultured bacteria were inoculated into 100 mL fresh FT or TSB medium containing 0, 50, 200, and 800 μg L^{-1} Cd at the OD_{600} value of ~0.1. Then, *A. muciniphila* was cultured for 48 h at 37 °C, while *B. pseudolongum* (37 °C) and *Psychrobacter* sp. (28 °C, 150 rpm) were cultured for 24 h to reach the stationary phases. At various time intervals during the culture, culture suspension samples were collected and detected for OD_{600}. At the end of culture, the culture suspension was centrifuged (10,000 rpm, 15 min, 4 °C) to separate bacteria from the culture medium. The medium was tested for Cd concentration by ICP-MS. The retrieved bacteria were washed, freeze-dried, and analyzed for Cd concentration using ICP-MS after digestion according to USEPA Method 3050B. Cadmium subcellular fractionation to Cd adsorbed on the cell wall, combined with peptidoglycan, existed inside the cells (cytoplasm), and existed on cell membranes was assessed (Kumar and Upreti, 2000). The recoveries of the Cd subcellular fractionation for *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. were 73.5 ± 10.9 %, 70.8 ± 12.1 %, and 76.6 ± 9.31 %, respectively. Details are provided in the SI. To evaluate the varied Cd immobilization ability of probiotics, metallothionein (MT) in three probiotic cells was measured using a microorganism ELISA Kit (MM-0941O1; Jiangsu Meimian Industrial Co., Ltd., China) as there were positive relations between immobilization ability of bacteria and the expression of MT (Zhang et al., 2024).

2.9. Data Processing and QA/QC

All data were presented as the mean and standard deviation of replicate analyses ($n = 6$). During the analysis using ICP-MS and ICP-OES, three measurements were conducted for each sample, with a relative standard deviation of <0.5 %. The stability of ICP-MS analyses was monitored by the indium isotope (^{114}In), with recoveries being 95 %–105 %. During mouse sample digestion, a certified rice standard reference material (GSB-21, Chinese Geological Reference Materials) was included, with the measured Cd concentration (0.012 ± 0.002 μg g^{-1}) comparable to the certified value (0.012 ± 0.003 μg g^{-1}). One-way ANOVA with Tukey's was conducted to test significant difference in tissue metal or mineral nutrition concentration among treatments ($p < 0.05$). Graphs were created using GraphPad Prism (version 9.0; GraphPad Software, Inc.).

3. Results and discussion

3.1. Mouse Growth and Gut Microbiota

In this study, mice were exposed to Cd-contaminated TL rice for 14 d, both with and without the daily intake of three strains of gut probiotics. Compared to mice fed Cd-free NJ rice (negative control), those exposed to TL rice, regardless of probiotic supplementation, consumed a similar amount of rice (~40 g) over the 14-d period (Fig. S1A). However, compared to mice fed only TL rice (Cd control), groups treated with *A. muciniphila* and *B. pseudolongum* exhibited notably higher body weight gain (Fig. S1B), suggesting benefits for mouse growth. This increase in body weight may be associated with the role of probiotic administration in promoting gut health.

To test this hypothesis, the gut microbiota of mice from the negative control, Cd control, Cd + A, Cd + B, and Cd + P groups was characterized. Across all groups, the Shannon diversity indices were comparable, indicating stable gut microbial diversity (Fig. S2A). However, principal coordinates analysis (PCoA) based on Bray-Curtis distance revealed clear separations between the negative control and Cd control groups, as well as between the Cd control mice and those receiving probiotics (Fig. S2B). These findings highlight the significant impacts of rice-Cd exposure on gut microbiota composition and the modulatory effects of probiotic administration.

Generally, oral administration of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. did not significantly enhance the growth of these target probiotics in the gut of mice (Fig. 1A). The low colonization rates of these probiotics may be attributed to their reduced survival when passing through the acidic stomach environment. However, despite their limited survival, these probiotic bacteria may still bolster intestinal health by promoting the growth of various beneficial bacteria and inhibiting harmful bacteria through their reactive proteins (Hu et al., 2021). Compared to the Cd control mice (2.06 %), the relative abundance of *Lactobacillus* in the Cd + A, Cd + B, and Cd + P groups was notably increased, averaging 4.54 %, 8.08 %, and 6.03 % respectively (Fig. 1B). Additionally, administration of *A. muciniphila* and *B. pseudolongum* significantly raised the relative abundance of *Bifidobacterium* from 0.20 % to 0.78 % and 0.71 % (Fig. 1B). The relative abundance of *Oscillospiraceae*, *Lachnospiraceae_NK4A136*, *Faecalibaculum*, and *Muribaculaceae* also tended to increase with probiotic administration, although these increases were not statistically significant (Fig. S3A).

Conversely, the relative abundance of *norank_f_Desulfovibrionaceae* decreased from 3.67 % to 1.98 %, 0.88 %, and 1.06 %, while that of *Helicobacter* reduced from 9.30 % to 5.11 %, 7.06 %, and 5.41 % following administration of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. (Fig. 1C). A similar trend was observed in the community of *Alistipes* (decreasing from 2.57 % to 1.13 %, 1.31 %, and 1.42 %) and *norank_o_Clostridia_UCG-014* (decreasing from 3.24 % to 1.38 %, 1.69 %, and 1.53 %) (Fig. S3B). Recent studies have classified these bacteria as pathogens and potential pathogens (Chen et al., 2023; Sun et al., 2024; Tian et al., 2023).

3.2. Fecal Cd Elimination

Elevated fecal Cd concentrations (8.01 ± 0.98, 9.15 ± 1.65, 8.46 ± 0.85 μg g^{-1}) were observed following oral administration of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp., compared to the Cd exposure control group (6.85 ± 1.33 μg g^{-1}) (Fig. 2A). Cadmium in feces represents the portion of rice-Cd intake that is not absorbed (Liu et al., 2021). The results suggest that probiotic ingestion limited the bioavailability of Cd in rice to the intestine by promoting its excretion through feces. Notably, *B. pseudolongum* and *Psychrobacter* sp. also contributed to the conversion of exchangeable Cd into unexchangeable species in mouse feces, as evidenced by significantly lower fecal Cd concentrations in the 1 M $MgCl_2$ extractable fraction (Fig. 2B) and higher concentrations in other fractions, particularly the sodium acetate (pH = 5.0) and 0.04 M NH_2OH•HCl extractable fractions when these two strains were administered to mice (Fig. S4). Previous studies have highlighted the protective role of probiotics *L. plantarum* CCFM8610 and *L. plantarum* L67 in mitigating the harmful effects of Cd exposure (Liu et al., 2020; Song et al., 2016). Zhai et al. (2019) reported that probiotic administration facilitated Cd excretion via feces, although the underlying mechanism remains unclear.

Previous studies have demonstrated that functional groups such as hydroxyl (−OH), amino (N − H), and phosphate, primarily from proteins and polysaccharides, play a significant role in the sorption of Cd (Kurultak et al., 2022; Wang et al., 2020b). In this study, *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. exhibited Cd tolerance when incubated in a medium containing 50–800 μg L^{-1} Cd, with their growth profiles remaining consistent with those observed in Cd-free conditions (Fig. 2C). Upon reaching the stationary phase, these strains significantly reduced the Cd concentration in the culture medium by 55.2 %–67.2 %, 84.9 %–94.5 %, and 27.1 %–41.8 %, respectively, irrespective of the initial Cd concentration (Fig. 2D). Notably, *B. pseudolongum* was the most effective, as evidenced by a 1.94- and 25.6-fold higher Cd concentration in its cells (387 μg g^{-1}) compared to *A. muciniphila* (199 μg g^{-1}) and *Psychrobacter* sp. (14.5 μg g^{-1}) when incubated in a medium with 800 μg L^{-1} Cd (Fig. 2E). A predominant amount of Cd (72.4 % and

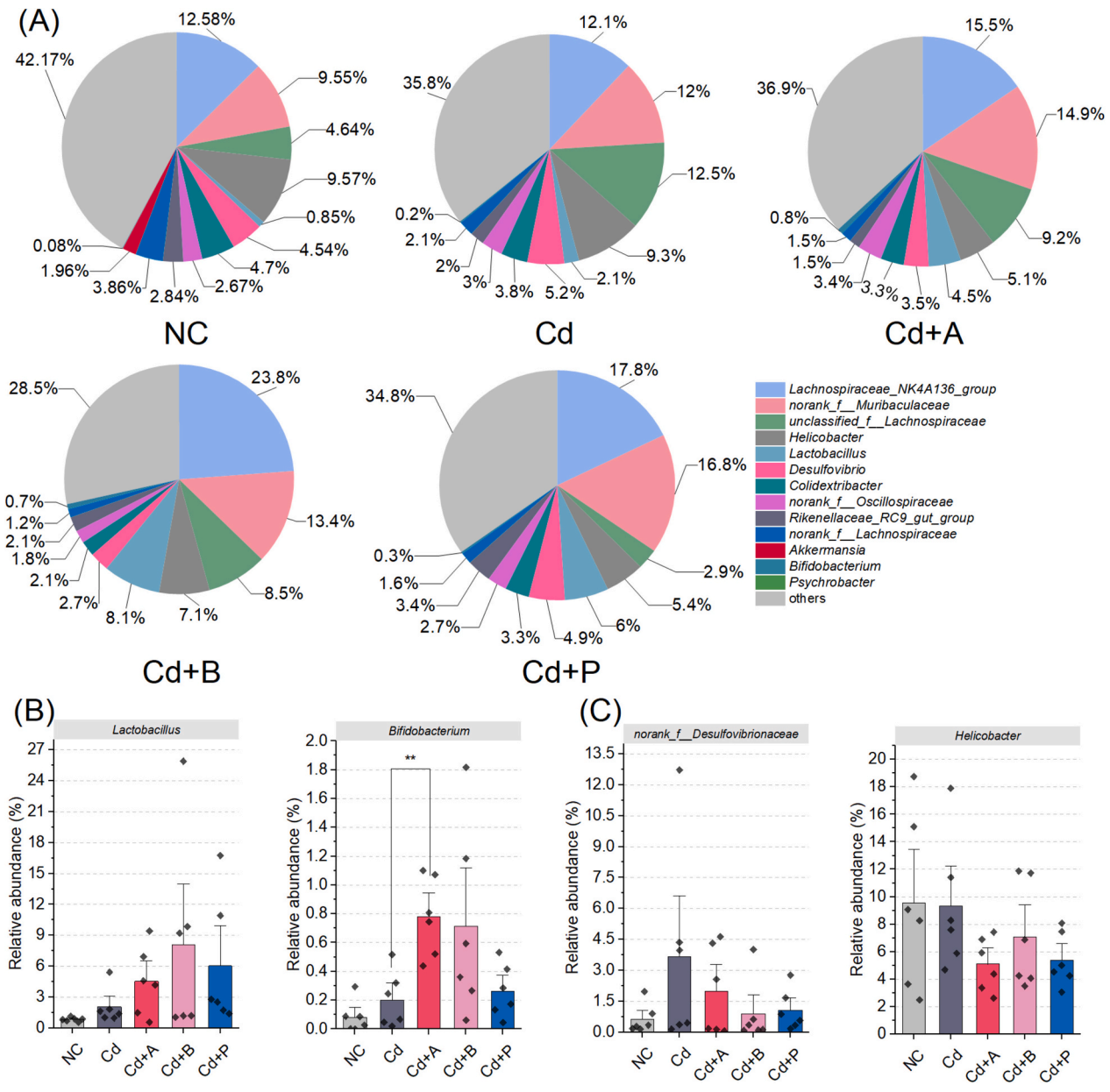


Fig. 1. Effect of intake of probiotics *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. on the composition of mouse gut microbiota under rice Cd exposure. (A) The composition profiles of gut microbiota at the genus level. (B) Relative abundance of *Lactobacillus* and *Bifidobacterium*. (C) Relative abundance of pathogens *Desulfovibrionaceae* and *Helicobacter*. NC: negative control mice without Cd exposure; Cd: Cd exposure control mice fed a Cd-contaminated rice containing 0.37 μg g^{-1}; Cd + A, Cd + B, and Cd + P: probiotics treated mice fed the Cd-contaminated rice and administrated daily with *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. at 5×10^8 CFU, respectively. **: $p < 0.01$.

75.4 %) was localized within the cells (cytoplasm and membrane) of *A. muciniphila* and *B. pseudolongum*, whereas for *Psychrobacter* sp., approximately 50 % of the total immobilized Cd was precipitated externally (adsorbed to cell walls and combined with peptidoglycan) (Fig. S5). Enhanced resistance and accumulation capacity towards Cd have been observed in recombinant *Escherichia coli* JM109 (pZH3–5/pMT) strain expressing the MT gene compared to the wild-type strain (Zhang et al., 2024). In this study, regardless of the presence or absence of Cd at 800 μg L^{-1} in the medium, the MT contents in *A. muciniphila* and *B. pseudolongum* were significantly higher than in *Psychrobacter* sp. (Fig. S6), likely contributing to their superior Cd immobilization efficiency. Despite varied Cd immobilization capacities, the *in vitro* results suggest that probiotics may facilitate Cd immobilization in the intestine, leading to increased Cd excretion via feces.

3.3. Tissue Cd Concentration

Surprisingly, although probiotics administration facilitated fecal Cd excretion, there were no significant decreases in Cd concentrations in livers (0.25 ± 0.023, 0.24 ± 0.036, and 0.28 ± 0.072 μg g^{-1}) and kidneys (1.50 ± 0.38, 1.52 ± 0.12, and 1.52 ± 0.42 μg g^{-1}) for mice administrated with *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. compared to Cd control mice (0.27 ± 0.055 and 1.74 ± 0.48 μg g^{-1}) (Figs. 3A, B).

To elucidate the mechanisms involved, we evaluated the body mineral nutrition status of mice, as it significantly influences the efficiency of intestinal Cd transport (Martinez-Finley et al., 2012). Surprisingly, a decrease in Ca concentration in mouse kidneys from 210 ± 7.67 to 184 ± 17.09 and 183 ± 11.47 μg g^{-1} was observed following administration of *B. pseudolongum* and *Psychrobacter* sp. (Fig. 3C). Iron concentrations in

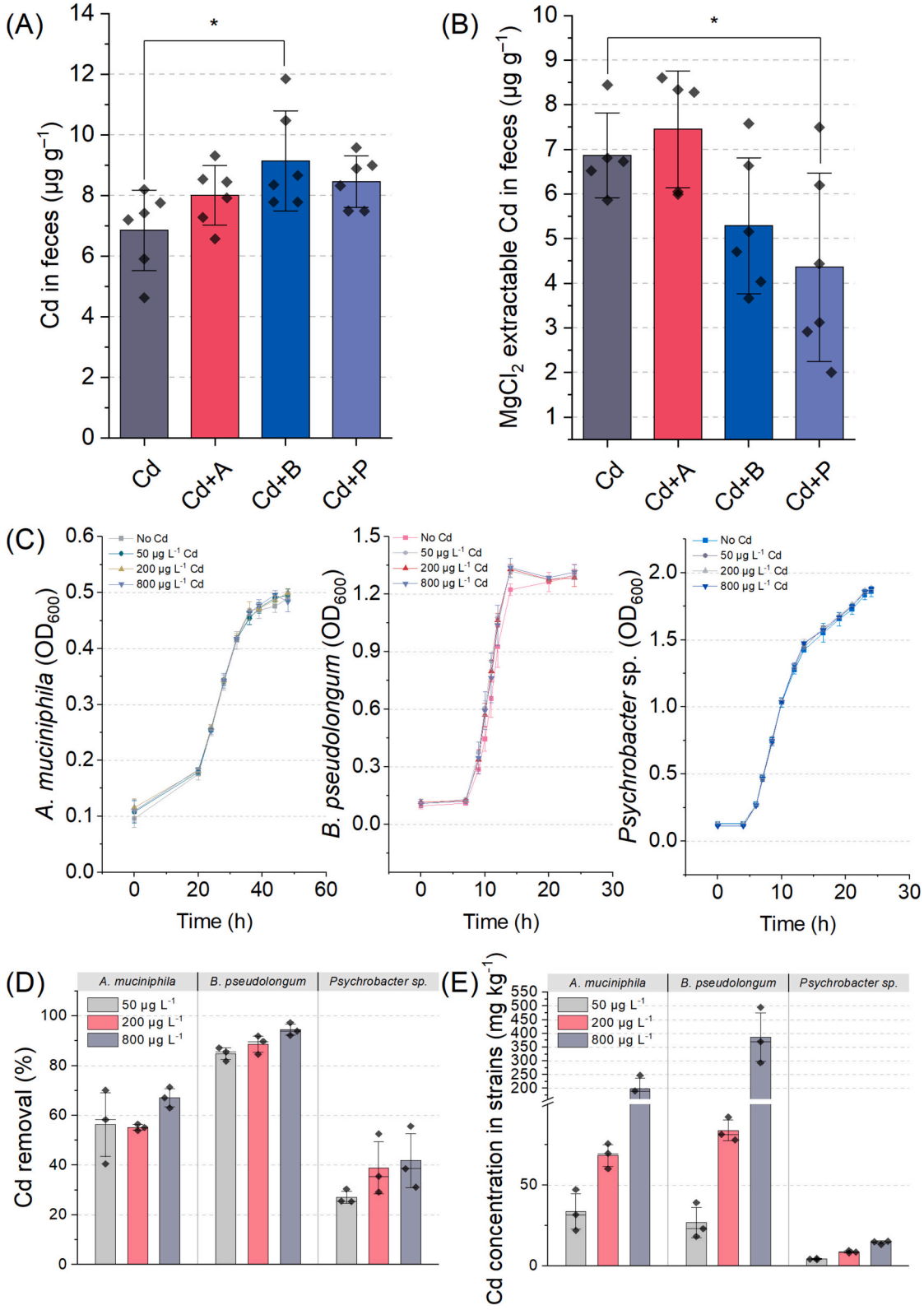


Fig. 2. The Cd immobilization ability of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. in the intestine and culture medium. (A) Cd concentration in feces for the groups of mice exposed to a Cd-contaminated rice containing 0.37 μg g^{-1} with or without *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. administration. Cd: Cd exposure control mice fed a Cd-contaminated rice containing 0.37 μg g^{-1}; Cd + A, Cd + B, and Cd + P: probiotics treated mice fed the Cd-contaminated rice and administrated daily with *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. at 5×10^8 CFU, respectively. (B) The change of $MgCl_2$ extractable Cd in feces of mice with probiotics administration. (C) The growth curves of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. in the culture medium containing 0, 50, 200, 800 μg L^{-1} Cd. (D) Reduction in Cd concentration in the culture medium after growth of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. with 50, 200, 800 μg L^{-1} Cd to reach the stationary phases. (E) Cd concentration in cells of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. when they were grown in the culture medium containing 0, 50, 200, 800 μg L^{-1} Cd. *: $p < 0.05$.

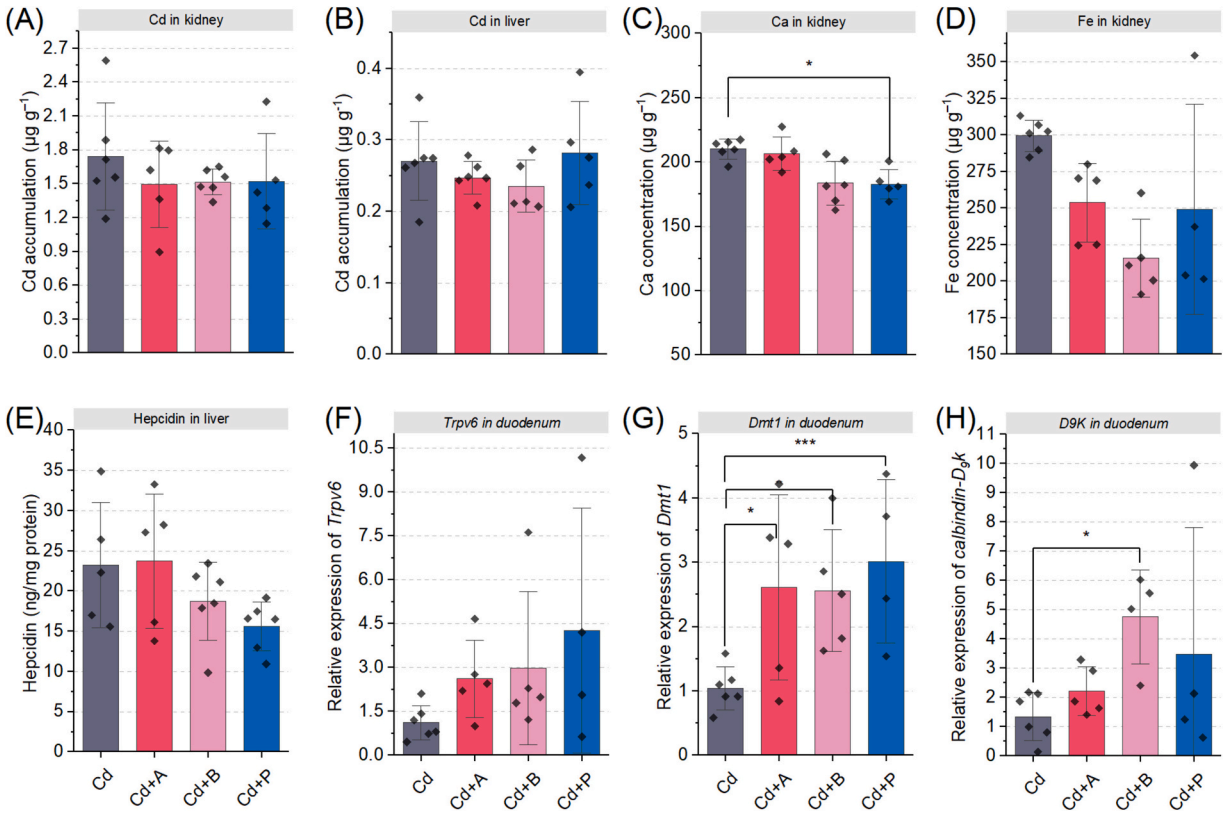


Fig. 3. Effect of intake of probiotics *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. on Cd, Ca, and Fe accumulation in mouse tissue and intestinal expression of genes encoding for Ca and Fe transporters under rice Cd exposure. (A, B) Changes of Cd concentration in mouse kidneys and livers with probiotics. (C, D) Reduction in Ca and Fe in mouse kidneys with probiotics administration. (E) Influence of probiotics administration on the hepcidin level in liver. (F—H) Changes in duodenal mRNA relative expression of apical Ca^{2+} and Fe^{2+} influx channel TRPV6 and DMT1 and intracellular Ca^{2+}-binding buffering protein D9K. Cd: Cd exposure control mice fed a Cd-contaminated rice containing 0.37 μg g^{-1}; Cd + A, Cd + B, and Cd + P: probiotics treated mice fed the Cd-contaminated rice and administrated daily with *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. at 5 × 10^8 CFU, respectively. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

the kidneys of groups receiving the three probiotic strains also decreased by 15.3–28.0 % compared to the Cd exposure control group, although only the group receiving *B. pseudolongum* exhibited a significant decrease (Fig. 3D). The Fe deficiency induced by probiotic treatment was further evidenced by a reduction in hepcidin levels in the mouse liver by 19.2–32.8 % with supplementation of *B. pseudolongum* and *Psychrobacter* sp., relative to the Cd exposure control group (Fig. 3E). Research has demonstrated that hepcidin production decreases during Fe deficiency to enhance intestinal Fe transport and maintain Fe homeostasis (Collins et al., 2018).

The findings contrast with reports suggesting that probiotics promote the intestinal absorption of minerals (Bielik and Kolisek, 2021). When exposed to Cd-contaminated TL rice containing only 133 μg g^{-1} Ca and 4.89 μg g^{-1} Fe, the administered strains of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp., as well as other probiotics stimulated by these strains, may compete with the host for the limited Ca and Fe available in the intestine (Ma et al., 2023), thereby reducing mineral bioavailability to the host. Probiotics also require minerals for normal growth. This requirement was evidenced by the detection of 950, 380, and 734 μg g^{-1} Ca and 358, 88.4, and 377 μg g^{-1} Fe (on a dry weight basis) in *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp., respectively (Fig. S7).

In response to body Ca and Fe deficiencies, transporters responsible for transcellular intestinal Ca^{2+} and Fe^{2+} transport are often upregulated to enhance Ca and Fe absorption and maintain mineral homeostasis (Collins et al., 2018; Kiela and Ghishan, 2018). Similar responses were observed in this study. Compared to the Cd exposure control group, the mRNA expression of the apical Ca^{2+} and Fe^{2+} influx channels TRPV6 and DMT1, and the intracellular Ca^{2+}-binding buffering protein D9K, was 2.33–3.08-, 2.45–2.88-, and 1.64–3.51-fold higher, respectively, in mice administered with probiotics (Figs. 3F-H). Consequently, transcellular Cd transport efficiency in the intestine may be significantly enhanced, as these mineral transporters also facilitate Cd absorption. This mechanism could substantially counteract the role of probiotic treatment in immobilizing Cd, resulting in no significant net decrease in Cd accumulation in mouse tissue under conditions of mineral deficiency.

3.4. Probiotics, Ca, and Fe Co-Intake

Given these findings, we further investigated whether co-treatment with probiotics, Ca, and Fe could effectively reduce Cd accumulation in mouse tissue by providing additional Ca and Fe to mitigate the competition between probiotics and the host. As anticipated, the administration of probiotics in conjunction with Ca and Fe resulted in 29.9–35.8 % and 145–198 % higher concentrations of Ca and Fe, respectively, in mouse liver, and 24.2 %–31.2 % higher Ca in the kidneys compared to the Cd exposure control mice (Fig. S8). With stable body Ca and Fe levels, the duodenal expression of genes encoding TRPV6, D9K, and DMT1 in mice receiving co-supplementation was maintained at levels similar to or even lower than those in the Cd exposure control groups (Fig. S9). Consequently, this did not counteract the Cd immobilization effects of probiotics, resulting in a reduction of Cd accumulation in the kidneys of the Cd + CaFe+A, Cd + CaFe+B, and Cd + CaFe+P groups by 63.2 %, 60.0 %, and 72.0 %, respectively, compared to the Cd exposure control group, although the co-supplementation did not reduce the Cd concentration in the liver (Figs. 4A, B). Interestingly, Ca and Fe ingestion alone also significantly reduced Cd concentration in mouse kidneys by 51.1 %; however, compared to Cd + CaFe, Cd accumulation

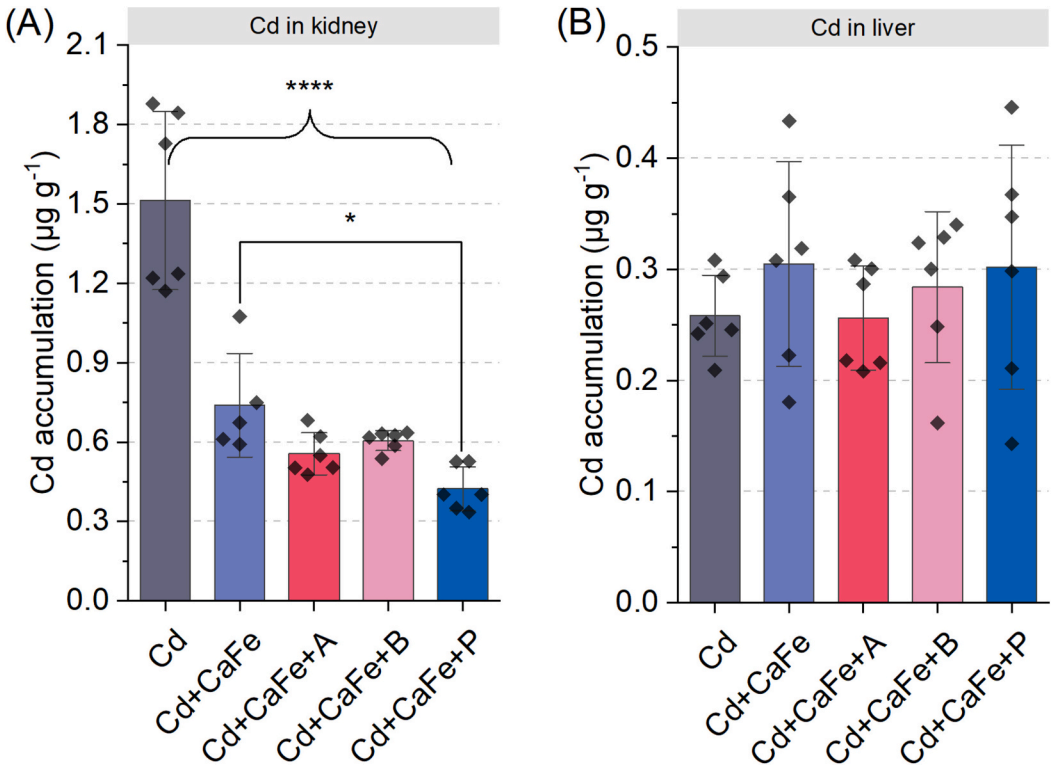


Fig. 4. The influence of probiotics, Ca, and Fe co-supplementation on Cd accumulation in mouse kidney (A) and liver (B) under rice Cd exposure. Cd: Cd exposure control mice fed a Cd-contaminated rice containing 0.37 μg g^{-1}; Cd + CaFe: Ca and Fe treated group fed $CaCO_3$-amended TL rice (500 μg g^{-1} Ca) and receiving water containing Fe (50 mg L^{-1} Fe); Cd + CaFe+A, Cd + CaFe+B, and Cd + CaFe+P: probiotics and mineral co-treated groups fed $CaCO_3$-amended rice TL, receiving water containing Fe, and daily administered with *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. via gavage, respectively. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

in the kidneys of the Cd + CaFe+A, Cd + CaFe+B, and Cd + CaFe+P groups was further reduced by 18.1–43 %. This indicates that under conditions of adequate Ca and Fe, the intake of probiotics effectively reduces Cd accumulation in mouse tissue, highlighting a promising strategy of daily co-intake of probiotics and essential minerals at adequate doses to reduce dietary Cd exposure in humans.

3.5. Intestinal Inflammation Risk

Although ineffective in reducing Cd accumulation in mouse tissue, administration of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. alone resulted in a 56.0–65.3 %, 58.2–69.6 %, and 50.9–92.0 % decrease in the ileal expression of *Il-1β*, *Il-6*, and *Tnf-α*, respectively, suggesting a reduced risk of intestinal inflammation (Figs. 5A-C). Similarly, serum IL-1β levels decreased by 15.6–12.6 % in the *A. muciniphila* and *B. pseudolongum* groups compared to the Cd control (Fig. 5D). This anti-inflammatory effect is largely attributed to the role of probiotics in enhancing gut health by promoting the growth of beneficial bacteria and inhibiting harmful bacteria (Fig. 1). Probiotics play a crucial role in maintaining the intestinal barrier and reducing chronic intestinal inflammation by strengthening intercellular tight junctions (Kang et al., 2022; Orlando et al., 2018), which helps prevent microbial contamination of interstitial tissues (Lynn et al., 2020; Marchiando et al., 2010; Vasileva et al., 2022). In this study, the relative expression of *Zo-1* was significantly upregulated in the Cd + P group compared to the Cd control group (1.71 vs 1.02), and *Psychrobacter* sp. administration enhanced *Claudin-4* expression (from 1.03 to 2.72) (Figs. 5E, G). For the Cd + A group, the relative expression of *Claudin-1*, *Claudin-4*, and *Cingulin* was upregulated, although not significantly (1.03 vs 2.30, 1.03 vs 1.89, 1.03 vs 2.21) (Figs. 5F-H).

3.6. Health Implications

Currently, the health of vast populations is threatened by elevated Cd concentrations in rice, a result of widespread soil Cd contamination (Shi et al., 2020). This health issue is expected to worsen under future climate change scenarios, as Cd concentrations in rice grown in contaminated soils are projected to increase significantly due to elevated atmospheric CO_2 and temperature (Guo et al., 2011), while the nutritional content of cereals may be reduced by approximately 10 % (Myers et al., 2015; Myers et al., 2014; Zhu et al., 2018). This scenario is likely to lead to heightened Cd exposure risks under climate change conditions. Without effective strategies to mitigate the impacts of climate change, diseases associated with Cd exposure, such as all-cause and cardiovascular mortality, bone and kidney diseases, and even cancers, are expected to become more prevalent (Satarug and Moore, 2004; Tellez-Plaza et al., 2012). Encouragingly, our findings indicate that Cd bioavailability in rice can be reduced by 60.0 %–72.0 % through the co-supplementation of probiotics, Ca, and Fe (Fig. 4), significantly reducing Cd exposure from rice consumption. Based on the data from this study, we recommend the daily use of probiotics in combination with Ca and Fe as an innovative approach to address the rice Cd and health crisis due to the two-sided effects of probiotics on the accumulation of Cd in tissues. On the one hand, probiotics can immobilize and adsorb Cd, converting the exchangeable Cd in the intestine into forms that are difficult for mice to absorb. On the other hand, probiotics compete for the absorption or utilization of mineral elements, which may lead to a state of nutrient deficiency in mice and, consequently, promote Cd accumulation. Evidently, a significant decrease in mineral elements (Ca, Fe, K, Mg) in the livers or kidneys can be observed, which aligns with the over-expression of Ca and Fe transporters (TRPV6, DMT1, and D9K) in the duodenum and the lower level of hepcidin in the liver of the groups supplemented with probiotics. Supplementing probiotics along with Ca

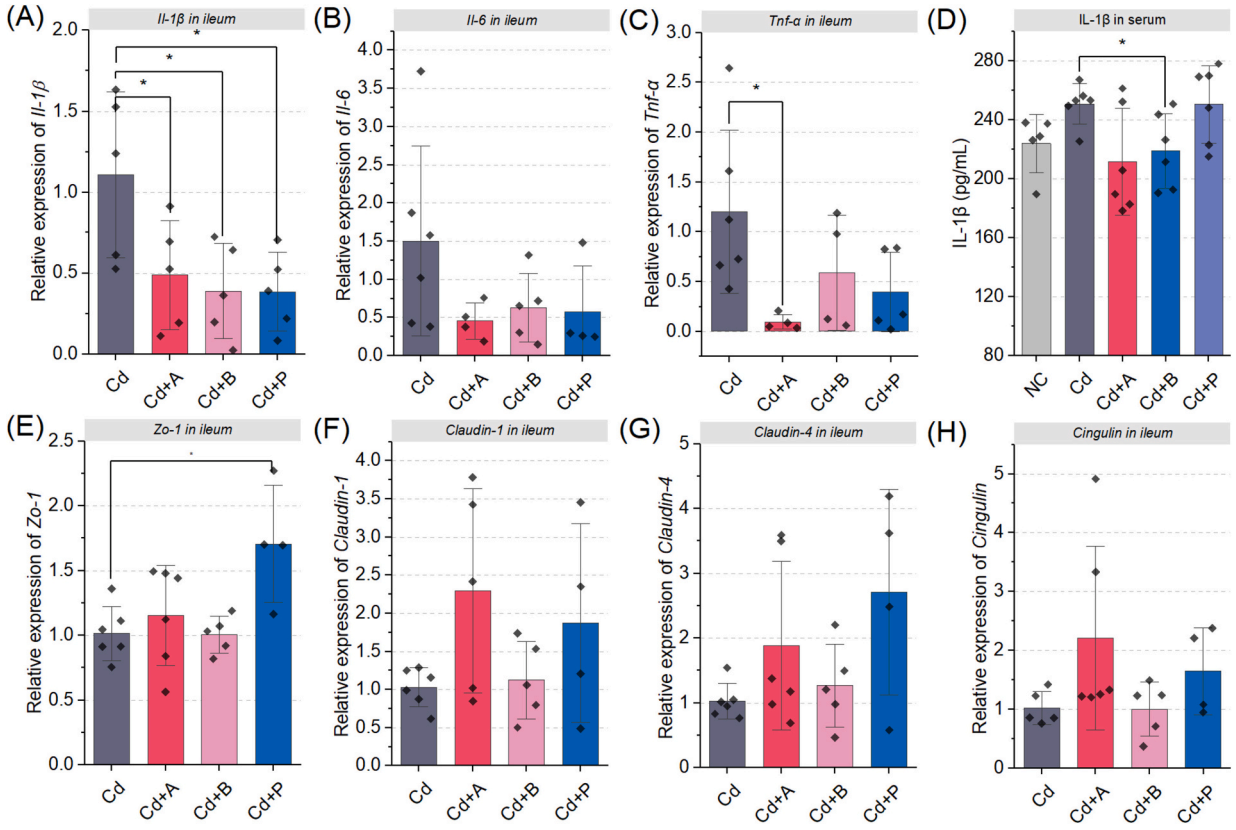


Fig. 5. The ability of *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. on alleviating the intestinal toxicity induced by Cd exposure. (A–C) Changes in mRNA relative expression of inflammatory mediators including *interleukin-1β (Il-1β)*, *Il-6*, and *Tumour necrosis factor-α* (*Tnf-α*) in mouse ileum with probiotics administration. (D) Serum IL-1β level in mice under Cd exposure with or without probiotics administration. (E–H) Changes in mRNA relative expression of genes encoding for intestinal tight junctions (TJs) including *Zo-1*, *Claudin-1*, *Claudin-4*, and *Cingulin* in mouse ileum with probiotics administration. Cd: Cd exposure control mice fed a Cd-contaminated rice containing 0.37 μg g^{-1}; Cd + A, Cd + B, and Cd + P: probiotics treated mice fed the Cd-contaminated rice and administrated daily with *A. muciniphila*, *B. pseudolongum*, and *Psychrobacter* sp. at 5 × 10^8 CFU, respectively. *: $p < 0.05$.

and Fe contributes to a significant decrease in Cd accumulation, and the up-regulated expression of *Trpv6*, *D9k*, and *Dmt1* recovers to a similar level as during Cd exposure alone. These results validate our hypothesis, demonstrating that while probiotics alone have a positive effect on inflammatory response, perturbation of gut microbiota, and intestinal injury induced by Cd exposure, concurrent supplementation of probiotics with Ca and Fe is an even more effective approach to reduce dietary Cd exposure risks.

Administering Ca and/or Fe alone at high concentrations can reduce rice Cd bioavailability (Zhao et al., 2017). However, long-term excessive intake of micronutrients can lead to metabolic imbalance, resulting in physiological abnormalities and even diseases. For example, long-term excessive Fe intake can result in chronic poisoning, damaging the liver, spleen, and other internal organs (Ma et al., 2023). Combined probiotics and micronutrient supplementation proves superior by mitigating some health risks associated with inappropriate doses or long-term Ca and Fe supplementation. This is evidenced by the significantly reduced diet consumption and loss of body weight in mice from the Cd + CaFe group compared to the Cd control group in this study, while the adverse effects of Ca and Fe intake were substantially mitigated by the administration of probiotics, particularly with *A. muciniphila* (Fig. S10).

Beyond lowering rice Cd bioavailability, the probiotic strategy significantly enhances gut health by increasing the abundance of probiotics in the gut, enhancing intestinal barrier function, and alleviating inflammatory responses (Figs. 1, 5). This approach may thus eliminate diseases associated with intestinal dysbiosis. The dual benefits of this strategy offer valuable insights into developing effective methods to manage rice Cd exposure and control diseases linked to Cd and other contaminants. Selenium-enriched probiotics have been demonstrated to alleviate colitis (Hu et al., 2022), regulating gut microbiota and host metabolism (Wang et al., 2024). Similarly, it would be worthwhile to explore the health benefits of Ca- and Fe-enriched probiotics in the future.

This study has inherent limitations that warrant further exploration. Firstly, the target probiotic strains did not successfully colonize the intestine, likely due to destruction by the acidic stomach environment. Consequently, a significant challenge is to develop methods that effectively deliver these probiotics to their intended location and ensure their survival such as probiotic embedding and extending the duration of probiotics supplementation. Secondly, the strategy of mineral supplementation involved the concurrent administration of Ca and Fe, without assessing the individual effects of each mineral. Additionally, determining the optimal doses of Ca and Fe is crucial for translating this theoretical approach into practical applications. Thirdly, given the proliferation of probiotics in various product formulations, it is essential to compare the efficacy of commercial probiotic products and identify the most effective formulation. Finally, it remains to be clarified whether varying amounts of probiotics administered significantly influence Cd bioavailability, necessitating further studies.

CRediT authorship contribution statement

Jin-Feng Xi: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. **Lei Zhou:** Supervision. **Yao-Sheng Zhang:**

Supervision. **Xin-Ying Lin:** Visualization. **Shan Chen:** Supervision. **Rong-Yue Xue:** Supervision. **Dongmei Zhou:** Supervision. **Hong-Bo Li:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology.

Declaration of competing interest

The authors declare no competing financial interest.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (42377429), the Fundamental Research Funds for the Central Universities (0211/14380219), and the Key Technologies Research and Development Program (2023YFC3708105).

Appendix A. Supplementary data

Gene primers (Table S1), diet consumption and body weight change of mice with administration of probiotics alone, gut microbiota α diversity and PCoA analysis of community structures, relative abundances of certain gut bacteria, Cd fractionation in mouse feces, Cd subcellular fractionation in cells of probiotics, MT, Ca, and Fe concentration in probiotics, and Ca and Fe concentration in mouse tissues, mRNA expression of Ca and Fe transporters, and diet consumption and body weight change of mice under probiotics, Ca, and Fe co-supplementation (Figs. S1–S10). Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177997.

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