# Zn-Enriched *Bacillus cereus* Alleviates Cd Toxicity in Mirror Carp (*Cyprinus carpio*): Intestinal Microbiota, Bioaccumulation, and Oxidative Stress

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## Abstract

Cadmium (Cd) poisoning in humans and fish represents a significant global problem. *Bacillus cereus* (*B. cereus*) is a widely used probiotic in aquaculture. The objective of this study was to evaluate the potential of *B. cereus* in ameliorating Cd-induced toxicity in mirror carp. The biosorption rate of Zn for the *B. cereus* in 85.99% was significantly more than five strains. All fishes were exposed for 30 days to dietary $ZnCl_2$ (30mg/kg), waterborne Cd (1 mg/L), and/or dietary Zn-enriched *B. cereus* (Zn 30mg/kg and $10^7$ cfu/g *B. cereus*). At 15 and 30 days, the fishes were sampled, and bioaccumulation, antioxidant activity, and intestinal microbiota were measured. Waterborne Cd exposure caused marked alterations in the composition of the microbiota. Dietary supplementation with Zn-enriched *B. cereus* can reduce the changes in the composition of intestinal microbiota in Cd exposure and decrease the pathogenic bacteria of *Flavobacterium* and *Pseudomonas* in Zn-enriched *B. cereus* groups. The results obtained indicate that Zn-enriched *B. cereus* can provide a significant protective effect on the toxicity of cadmium by inhibiting alterations in the levels of bioaccumulation and antioxidant enzyme including superoxide dismutase (SOD), catalase (CAT), total antioxidant (T-AOC), and malonaldehyde (MDA). Our results suggest that administration of Zn-enriched *B. cereus* has the potential to combat Cd toxicity in mirror carp.

**Keywords** *Bacillus cereus* · Cadmium · Intestinal microbiota · Accumulation · Antioxidant stress

## Introduction

Heavy metals with the unknown biological role persist in the natural environment and have the capacity to exert toxic effects at relatively low levels[1]. Cadmium (Cd) is utilized worldwide to facilitate many industrial applications. In addition, Cd, a toxic metal, has been recognized as one of the most deleterious heavy metal pollutants that severely threaten the health of the human and other organisms, particularly aquatic animals because it is not only readily assimilated at late in fish and in mammals along the food chain but it is highly toxic with a long biological half-time of 10–30 years [2]. As a nonessential biological element, Cd is widely toxic, affecting the intestinal microbiota and causing oxidative stress, even leading to death (at high concentrations) [3].

Cd can accumulate at relatively high levels in animals; it can also accumulate in fish upon occurrence of high levels of either waterborne or foodborne Cd [4]. The accumulation of Cd can induce overproduction of reactive oxygen species (ROS) in various organisms, and this overproduction results in oxidative stress [5]. Oxidative stress can also increase the hosts' susceptibility to damage by promoting the reactivity of ROS with proteins, lipids, and DNA [6]. In addition, gut microbiota plays a crucial role in remaining stable and beneficial on the host health, as they are involved in numerous important physiological, nutritional, immunologic, and metabolic processes [7, 8]. Many authors suggested that taking the adverse effect of heavy metals on the intestines into consideration as well, the gut microbiota, which lives in symbiosis with

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intestinal epithelial cells, could be affected by oral exposure to toxic metals [9, 10]. In previous reports, Cd exposure has been found to alter the composition and the microbial diversity of gut community [11].

Up to now, for mammals to humans, of the various available dietary supplements (e.g., essential metal, vitamins, edible plants, phytochemicals, and probiotics) [12–15], Zn and probiotics may potentially reduce the heavy metal toxicity. Zinc (Zn) is the second most abundant essential trace element for animals and humans, and it plays a role in numerous biological processes, such as cell proliferation and differentiation, signal transduction, neurodevelopment, and learning and memory [16]. Zn has been shown to reduce the oxidative damage brought about by free radicals. A few studies reported that Zn alleviated Cd toxicity in fish [17, 18]. Khan et al. reported that Zn-stimulated mucus secretion into the rainbow trout intestine inhibits Cd accumulation and Cd-induced lipid peroxidation [18]. In addition, Zn enrichment can influence the uptake and oxidative damage that is usually attributed to dietary Cd exposure [19]. Probiotics are living microorganisms that are beneficial to an individual's health, which can protect hosts against a variety of diseases including both bacterial and viral diarrhea [20]. Many reported that probiotics show a potential for alleviating heavy metal toxicity [20]. A few reported that *Lactobacillus plantarum* (*L. plantarum*) could reverse the alterations in the intestinal microbiota composition caused by heavy metal, alleviate oxidative stress in tissues, and relieve the changes in hematological parameters [12, 21]. Compared with *L. plantarum*, *Bacillus subtilis* is a kind of probiotic and can survive in low pH environments and wider temperatures, which were widely used in aquaculture [22]. Many authors suggested that *Bacillus*, including *Bacillus subtilis* or *Bacillus cereus*, can reduce Cd and Pb accumulation in organs, modulate intestinal microbiota, and change antioxidant activity following Cd or Pb exposure in *Carassius auratus gibelio* [11, 23, 24].

It has recently been demonstrated that probiotics can absorb or tolerate the micro-macro elements (e.g., Ca, Cu, and Zn) or toxic elements (e.g., Cd and Pb) [12, 25]. Many studies suggested that *L. plantarum* or *Bacillus* could tolerate Cd or Pb, which is one of the important foundations against heavy metal toxicity [11, 12, 21, 23]. However, few studies have investigated the effects of Cd and Zn-enriched *B. cereus* on accumulation in mirror carp. In this study, we screen the six strains of *Bacillus* in adsorption for Zn. Compared with the other five strains, *B. cereus* showed better ability in Zn adsorption. In addition, we investigated the effects of Cd and Zn-enriched *B. cereus* on intestinal microbiota, bioaccumulation, and oxidative stress in mirror carp. The results showed that Zn-enriched *B. cereus* dietary reduces the concentration Cd of tissues, modulates intestinal microbiota, and changes antioxidant activity following Cd exposure in mirror carp.

## Materials and Methods

### Bacterial Strain

Six *Bacillus* strains were used in this study, all of which were obtained from the gut of mirror carp or natural water in Jilin Province (China). This isolation is achieved by reference to the method used in previous studies [26]. Prior to use, the organism was cultured in Luria-Bertani (LB) medium at 30 °C for 18 h using an orbital shaker at 150 rpm under aerobic conditions.

### Biosorption of Trace Metal in the Liquid Medium

Six *Bacillus* strains (*B. cereus*, *B. subtilis*, *B. megaterium*, *B. amyloliquefaciens*, *B. pumilus*, and *B. licheniformis*) were cultured in LB medium containing $ZnCl_2$ (Sinopharm Chemical Reagent Company, Shanghai, China). The design of our experiment was based on the previous study [27]. The strains were exposed to 50mg/L of $ZnCl_2$, and biosorption was evaluated after 3 days of exposure. Briefly, after 24-h subculture at 30 °C on LB medium, cells were harvested off the ager surface (about 50-mg dried mass) and transferred to 50-mL LB medium at 180 rpm. After 12 h of cell growth, Zn was inserted from a pre-prepared stock solution. The control was prepared in the same condition without metal. After 24 h of exposure under these conditions (30°C, 180 rpm), cells were filtered on 0.22-μm membrane and gently rinsed with 50 mL of ultrapure water in order to liberate and collect the metal adsorbed on the fungal cell wall. At the end of the experiment, three new fractions were obtained: the liquid media (fraction A) and the dried mycelium (fraction B). Fraction A and fraction B were mineralized before the analysis of the trace metals. The Zn concentration in food was measured using an Atomic Absorption Spectrophotometer AA-6300 (Shimadzu, Japan). The percentage of absorbed mental was calculated as the quantity of metal absorbed inside the cells (A, fraction B) divided by the initial quantity of metal in the culture media (B): %A=A/B.

### Scanning Electron Microscope (SEM) Analysis

The scanning electron microscope was analyzed according to the method described by Wang et al [11]. Briefly, *Bacillus cereus* was cultured in Luria-Bertani (LB) medium at 30 °C for 12 h using an orbital shaker at 150 rpm under aerobic conditions, and then the cells were transferred to coverslip in 24-well plates in LB medium with 200-mg/L Zn at 30 °C for 4 h. Afterward, the cells were 2.5% (v/v) glutaraldehyde in 0.1 M PBS (pH 7.4) for 1 h at room temperature and then washed with PBS three times. The cells were dehydrated in a graded series of ethanol (30–100%), passed in 100% tertiary butanol (Life Technologies), and then dried in a vacuum

freeze dryer and coated with gold. The specimens were examined with a S-4800 Field Emission Scanning Electron Microscope (Hitachi).

## Diet Preparation

Mirror carp (45.84 ± 0.68 g; 15.1 ± 0.1 cm) were obtained from a specialized aquatic fry farm (Jilin Province, China) and transported to the laboratory. Commercial feed (crude protein 37.7%, crude lipid 7.4%, and ash 10.8%) is obtained from Jinyanhong Aquarium Products Co., Hangzhou, China, and was used as the basal diet. The fish adapted to laboratory conditions for 2 weeks before the exposure trial. The fish was granted with the commercial feed twice daily at a rate of 1–2% of body weight and sustaining experimental conditions (dissolved oxygen 6.59 ± 0.07 mg/L; pH 7.2 ± 0.3; ammonia less than 0.5 mg/L; nitrites less than 0.05 mg/L; water temperature 25 ± 2 °C; light:dark = 13h:11h). The concentration of *B. cereus* in the feed was determined by spread plate technique (nutrient agar incubated at 30 °C for 24 h). Experiments were performed according to the research protocols approved by the Institutional Animal Care and Use Committee, Jilin Agricultural University.

## Experimental Diets and Experimental Design

At the end of accommodation, 300 fishes were randomly divided into five groups with three replications each (fifteen fish per tank) and resided in 80-L plastic tanks. Waterborne Cd (1 mg/L) and/or dietary Zn-enriched *B. cereus* (including Zn 30-mg/kg and $10^7$-cfu/g *B. cereus*) and/or dietary $ZnCl_2$ (30mg/kg). The groups were distributed as follows: C0Z0B0 (control group), C0Z1B0 (Zn 30mg/kg), C0Z1B1 (Zn 30mg/kg and *B. cereus* $10^7$cfu/g), C1Z0B0 (Cd 1mg/L), and C1Z1B1 (Cd 1mg/L, Zn 30mg/kg, and *B. cereus* $10^7$cfu/g). During the excrement, the condition was the same as accommodation. The experiment lasted for 30 days. The control group was fed a basal diet with the same volume of sterile saline, and all food was stored at 4 °C until use. The Zn concentration in food was measured using an Atomic Absorption Spectrophotometer AA-6300 (Shimadzu, Japan). A total of 50-mL water samples were collected from fish tanks of different groups, and the Cd level was analyzed using the same method as tissues. The actual concentration of waterborne Cd and dietary-borne Zn was presented in Table 1. In addition, for steadying the Cd concentration in water, Cd was added according to the experimental concentration, and the water was substituted once a day with half a tank of water.

## Sample Collection

The fishes were fasting for 24 h before collecting samples. Six fishes were randomly selected from each tank for 15 and 30

days to collect tissue samples and blood samples. The fishes were euthanized with 300 mg/L of methanesulfonate 222 (MS-222). Tissue samples were then subsequently collected as spleen and gut. The blood samples were collected using a syringe treated with heparin and then separated by centrifugation at 4000 g for 5 min at 4 °C immediately. All samples were frozen in liquid nitrogen and stored at −80 °C until the experimental assays.

## Analysis of Gut Microbial Diversity

As very few gut contents could be collected from the fish in the groups, samples were pooled by tank (*n* = 3, each group). Genomic DNA was extracted from fecal samples using the E.Z.N.A. Soil DAN Kit (Omega Bio-tek, Norcross, GA, USA), following the standard protocol. The V3–V4 region of the bacterial 16S ribosomal RNA gene was amplified by PCR using the primers 338F 5′-ACTCCTACGGGAGGCAGCAG-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′, where the barcode is an eight-base sequence unique to each sample. The PCR reactions were performed in triplicate, and the mixture consisted of 10-ng template DNA, 2-μL dNTPs, 0.8-μL forward primer, 0.8-μL reverse primer, 4-μL 5 × FastPfu Buffer, 0.4-μL FastPfu Polymerase, and ddH2O in a final volume of 20 μL and the PCR amplification program according to the method described by Wang et al [28].

Amplicons were extracted from 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA). Then, purified amplicons were pooled and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. The raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17). Operational units (OTUs) were clustered with a 97% similarity cutoff using UPARSE. And chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed using the RDP Classifier against the SILVA (SSU115) 16S rRNA database using a confidence threshold of 70%.

## Cd Accumulation

Five tissues (gill, kidney, gut, liver, and muscle) from each group were sampled on days 15 and 30 to observe Cd concentration. Tissues were enclosed in a digestion vessel and mixed with 10 mL of concentrated nitric acid (65% $HNO_3$). The digestion reaction occurred from 60 °C to a finial temperature of 95 °C in the hot plate until the samples were completely dissolved. The clear liquid was diluted with demonized water to 20 mL and then assayed for concentration of Cd using an Flame Atomic Absorption Spectrophotometer AA-6300

**Table 1** Nominal and actual Zn and Cd concentration in water (mg/L)

| Groups | Nominal Cd concentration (mg/L) | Actual Cd concentration (mg/L) | Nominal Zn concentration (mg/kg) | Actual Zn concentration (mg/kg) |
|---|---|---|---|---|
| Control group | 0 | 0.006 ± 0.001 | 0 | 0.005 ± 0.001 |
| Zn group | 0 | 0.007 ± 0.012 | 30 | 29.452 ± 0.872 |
| Zn-enriched *B. cereus* | 0 | 0.004 ± 0.020 | 30 | 31.457± 1.031 |
| Cd group | 1 | 1.015 ± 0.011 | 0 | 0.006 ± 0.021 |
| Zn-enriched *B. cereus* plus Cd | 1 | 0.978 ± 0.014 | 30 | 29.459 ± 0.994 |

(Shimadzu, Japan) at minimum measured concentration of 1 μg/L (0.001 mg/L). In this study, the standard reference material is 99.99 % of Cd and Zn standard. In addition, the Cd concentration in organ is determined by a third-party testing agency (Pony Testing International Group Co, Ltd, China). Quality assurance and quality control are carried out in accordance with laboratory standards (ISO/IEC 17025/ ISO/IEC 17020).

### Antioxidant Response Analysis

The liver was studied for antioxidant activity on days 15 and 30 days of the feeding period. Superoxide dismutase (SOD), catalase (CAT), total antioxidant (T-AOC), and malonaldehyde (MDA) were determined using commercially available Elisa kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the method described by Dai et al [29].

### Statistical Analysis

All data were submitted to analysis of variance, ANOVA, followed by Tukey's test and using data analysis software SPSS 20.0 (SPSS, Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD) and differences with $P$ values of < 0.05 were considered to be statistically significant.

## Results

### Biosorption Experimentation and SEM Analysis

Table 1 shows the biosorption percentages measured under the different conditions of our experiment.

The biosorption rate of Zn for the *B. cereus* was 85.99% (729.83 mg/kg) on the LB medium, but only Zn concentrations as low as 76.77% (651.13 mg/kg), 69.18% (586.75), 67.7% (574.21 mg/kg), 70.25% (595.83 mg/kg), and 81.56% (691.75 mg/kg) were able to absorb completely *B. subtilis*, *B. megaterium*,

*B. amyloliquefaciens*, *B. pumilus*, and *B. licheniformis*. Compared with the other strain, *B. cereus* has shown the highest biosorption rate. The SEM result was indicated in Fig. 1. Compared with the control group (Fig. 1a), the cell morphology of *B. cereus* with Zn treatment showed a complete cell morphology and zinc adsorption particles Table 2.

### Gut Microbial Diversity

As shown in Fig. 2 and Table 3, gut microbial diversity in the gut during 30 days is presented. At a threshold of 97% sequence identity, a total of 1146 OTUs were identified in the present study. Briefly, mean of 814, 1013, 816, 925, and 963 OTUs were assigned to C0Z0B0, C0Z1B0, C0Z1B1, C1Z0B0, and C1Z1B1 groups (Fig. 2a). In the phylum (Fig. 2c) and genus (Fig. 2d) levels, both Cd exposure and *B. cereus* treatment altered the structure and composition of the fish gut microbiota. Compared with the control group, *Photobacterium* sp. was significantly decreased in other groups; *Flavobacterium*, uncultured_bacterium_f_Micrococcaceae, and *Pseudomonas* were significantly increased in C1Z0B0 and C1Z1B1 groups; *Cetobacterium* was significantly increased in C0Z1B0; uncultured_bacterium_f_Mycoplasmataceae was significantly decreased in C1Z0B0 and C1Z1B1; and uncultured_bacterium_f_Mycoplasmataceae was significantly increased in C0Z1B1.

### Cd Accumulation

Zn-enriched *B. cereus* significantly reduced the levels of the concentration of Cd in tissues (Fig. 3). Compared with the control group, accumulation of Cd in the tissues increased significantly following exposure to water-borne cadmium with a positive correlation to the concentration. In addition, compared with the Cd-only administration group, Cd concentration in all studied tissues was significantly decreased ($P < 0.05$) in the Zn-enriched *B. cereus* group.

**Fig. 1** The morphology of *B. cereus* by scanning electron microscope analysis: control (**a**) and 200 mg/L Zn (**b**)
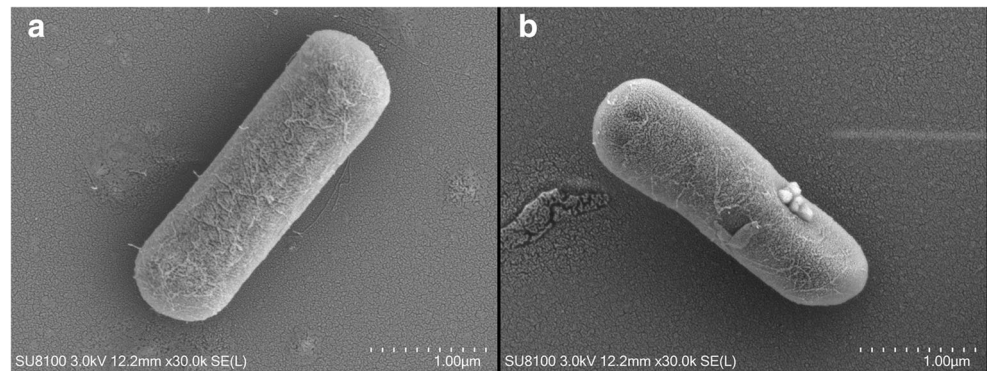


## Antioxidant Responses

The antioxidant responses to the liver of common carp was evaluated by measuring SOD, CAT, MDA, and T-AOC activity following exposure to waterborne Cd and/or dietary Zn-enriched *B. cereus* (Fig. 4).

Compared with the control group, SOD and CAT were significantly increased in the 30-mg/kg Zn and Zn-enriched *B. cereus* group, but significantly decreased in the Cd exposure group (1-mg/L Cd and Zn-enriched *B. cereus* plus Cd groups); however, MDA and T-AOC were significantly decreased in the 30 mg/kg Zn and Zn-enriched *B. cereus* group, but significantly increased in the Cd exposure group (1mg/L Cd). Compared with the only 1 mg/L Cd group, SOD and CAT were significantly increased in the Zn-enriched *B. cereus* plus Cd group; however, MDA and T-AOC were significantly decreased in the Zn-enriched *B. cereus* plus Cd group.

## Discussion

Fish is rich in multiple essential nutrients and proteins, which can also be easily digested and absorbed by human. However, as industrial development progresses, the general public is facing serious Cd pollution problems [30]. Due to the bioaccumulation, Cd was accumulated to higher levels in fish with

**Table 2** Six strains biosorption percentages and concentration after metal exposure

| Genus | Percentages (%) | Concentration (mg/kg) |
| --- | --- | --- |
| *Bacillus subtilis* | 76.77±1.84 | 651.13±15.61 |
| *Bacillus megaterium* | 69.18±1.32 | 586.75±11.19 |
| *Bacillus amyloliquefaciens* | 67.71±1.36 | 574.21±11.53 |
| *Bacillus pumilus* | 70.25±0.89 | 595.83±7.54 |
| *Bacillus cereus* | 85.99±0.45 | 729.83±3.81 |
| *Bacillus licheniformis* | 81.56±1.74 | 691.75±14.75 |

a risk for consumers [31]. In addition, Cd accumulation causes intestinal flora disorders and oxidative stress [10, 32]. Therefore, it is of importance to develop innovative dietary therapeutic strategies for the prevention and treatment of Cd toxicity. The aim of this study consisted of *Bacillus* strains with potential properties against Cd toxicity in common carp.

Probiotics, including *Bacillus* and lactic acid bacteria, are living microorganisms that are beneficial to an individual's health. It has been reported that lactic acid bacteria are effective in alleviating oxidative stress induced by several diseases. In addition, Zhai et al (2014) demonstrated that lactic acid bacteria have a strong cadmium tolerance and adsorption [25]. Apart from Cd tolerance and adsorption, lactic acid bacteria also have a strong lead, gastrointestinal properties, and perfluorooctanoate tolerance and adsorption [12, 25, 33]. *Bacillus* strains were widely used as a probiotic in aquaculture because it is able to tolerate a wide range of temperatures and low pH. In previous study, our data suggest that *Bacillus* can tolerate the higher concentration of Cd [32]. In addition, the minimal inhibitory concentration (MIC) of Cd for *B. cereus* was 300 mg/L on the LB medium (previous study). In this study, we screened six *Bacillus* for zinc adsorption and transformation ability. Interestingly, *B. cereus* also shows the strongest for Zn biosorbed in vitro. Meanwhile, the SEM result shows the same as adsorption result. The cadmium tolerance and zinc adsorption capacity of *B. cereus* in vitro provide a strong theoretical basis for reducing cadmium toxicity in vivo.

Gut microbiota play a role in remaining stable and beneficial on the host's health, as they are involved in numerous important physiological, nutritional, immunologic, and metabolic processes. Mu et al. suggested that Cd can induce a significant change in the intestinal microbiota composition of *Rana chensinensis* tadpoles [34]. Similar results were also observed in *Carassius auratus gibelio* and Nile tilapia [11, 35]. Previous reports have shown that there are four phyla of dominant bacteria in the intestinal tract of common carp (*Cyprinus carpio L.*): *Fusobacteria*, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* [36]. In this study, the dominant bacteria at the phylum level of mirror carp was
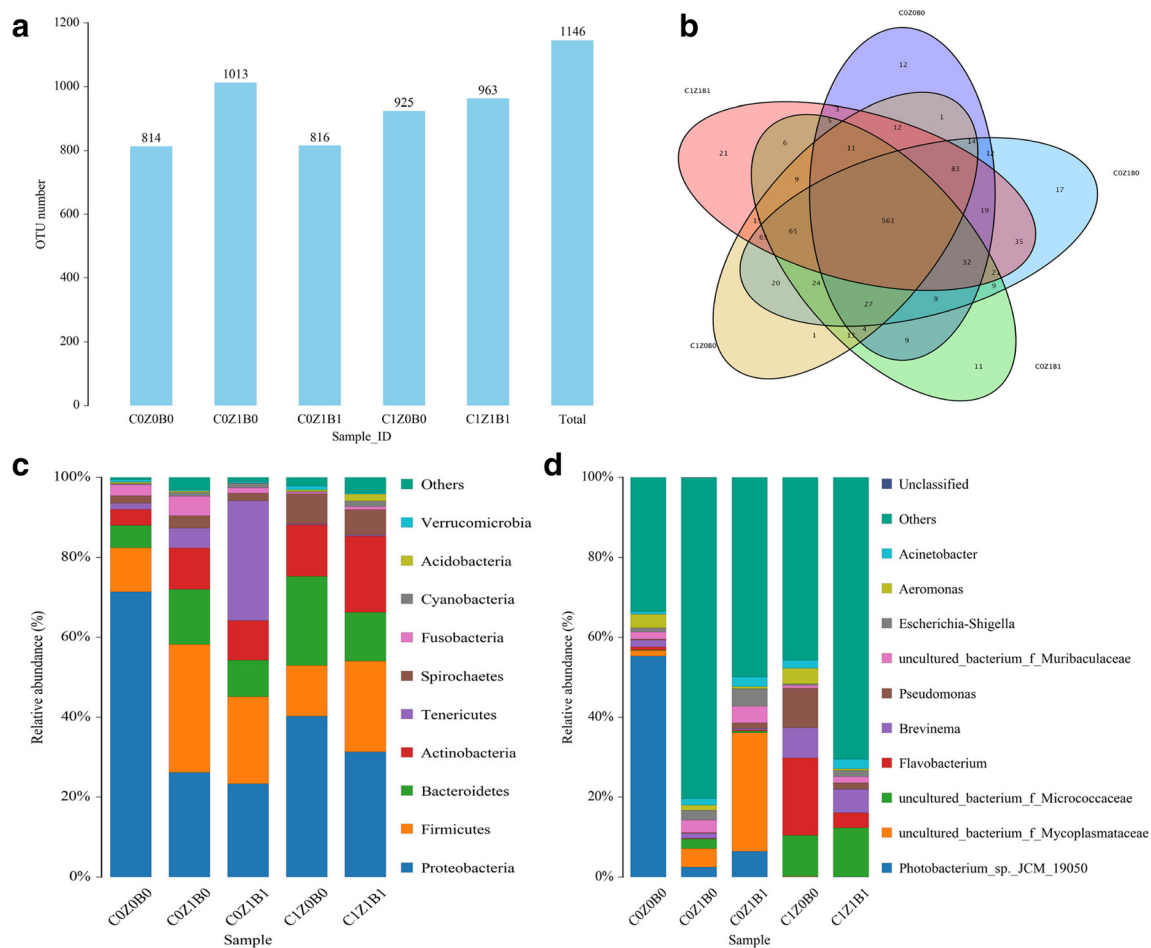
**Fig. 2** Five groups of gut microbiota in OTUs (**a**) and Venn (**b**); community bar-plot analysis shows relative abundance of gut microbiota in each group at the phylum level (**c**) and genera level (**d**). C0Z0B0 (control group), C0Z1B0 (Zn 30mg/kg), C0Z1B1 (Zn 30mg/kg and *B. cereus* $10^7$cfu/g), C1Z0B0 (Cd 1mg/L), C1Z1B1 (Cd 1mg/L, Zn 30mg/kg and *B. cereus* $10^7$cfu/g)

*Proteobacteria, Firmicutes, Actinobacteria,* and *Bacteroidetes*. In our previous study, Cd or *B. cereus* treatment was found to have significantly decreased the population of the *Proteobacteria* and considerably increased the *Firmicutes* at the phylum level in the gut of the *Carassius auratus gibelio* [11, 32]. Our results are consistent with previous studies at the phylum level in the gut of the common carp. Interestingly, *Photobacterium* sp. is the dominant

bacteria of common carp intestines. In this study, this phenomenon is distinct from other studies [10, 34]. This may be related to the environment in mirror carp. In addition, in this study, our data suggested that *Photobacterium* sp. was significantly decreased in all experimental groups compared with the control group. Currently, we have isolated *Photobacterium* sp. in the common carp intestine (data unpublished), and then the function of *Photobacterium* sp. will be

**Table 3** Relative abundance (%) of in the intestinal tissues of common carp following dietary Zn-enriched *Bacillus cereus* and waterborne Cd

| Genus | Groups | | | | |
|---|---|---|---|---|---|
| | CK | C0Z1B0 | C0Z1B1 | C1Z0B0 | C1Z1B1 |
| *Photobacterium* sp. JCM 19050*(%)* | 54.76±5.12[a] | 2.77±0.34[b] | 6.15±1.81[b] | 0.030±0.017[b] | 0.041±0.017[b] |
| *Flavobacterium (%)* | 0.77±0.59[a] | 0.12±0.04[a] | 0.23±0.19[a] | 19.31±1.71[b] | 3.72±0.32[c] |
| *Pseudomonas (%)* | 0.21±0.03[a] | 0.23±0.08[a] | 1.428±0.02[b] | 10.01±0.96[c] | 1.61±0.06[b] |
| *uncultured_bacterium_f_Mycoplasmataceae (%)* | 1.36±0.08[a] | 4.52±0.41[b] | 29.1±1.12[c] | 0.13±0.01[d] | 0.03±0.001[d] |
| *uncultured_bacterium_f_Micrococcaceae (%)* | 0.13±0.01[a] | 2.18±0.25[b] | 0.41±0.01[a] | 10.15±1.12[c] | 12.32±1.05[c] |
| *Cetobacterium (%)* | 2.14±0.44[a] | 4.31±0.45[b] | 1.91±0.12[a] | 0.32±0.01[c] | 1.31±0.61[a] |

Data are mean ± S.D. Values in rows with same superscript does not differ significantly ($P > 0.05$)
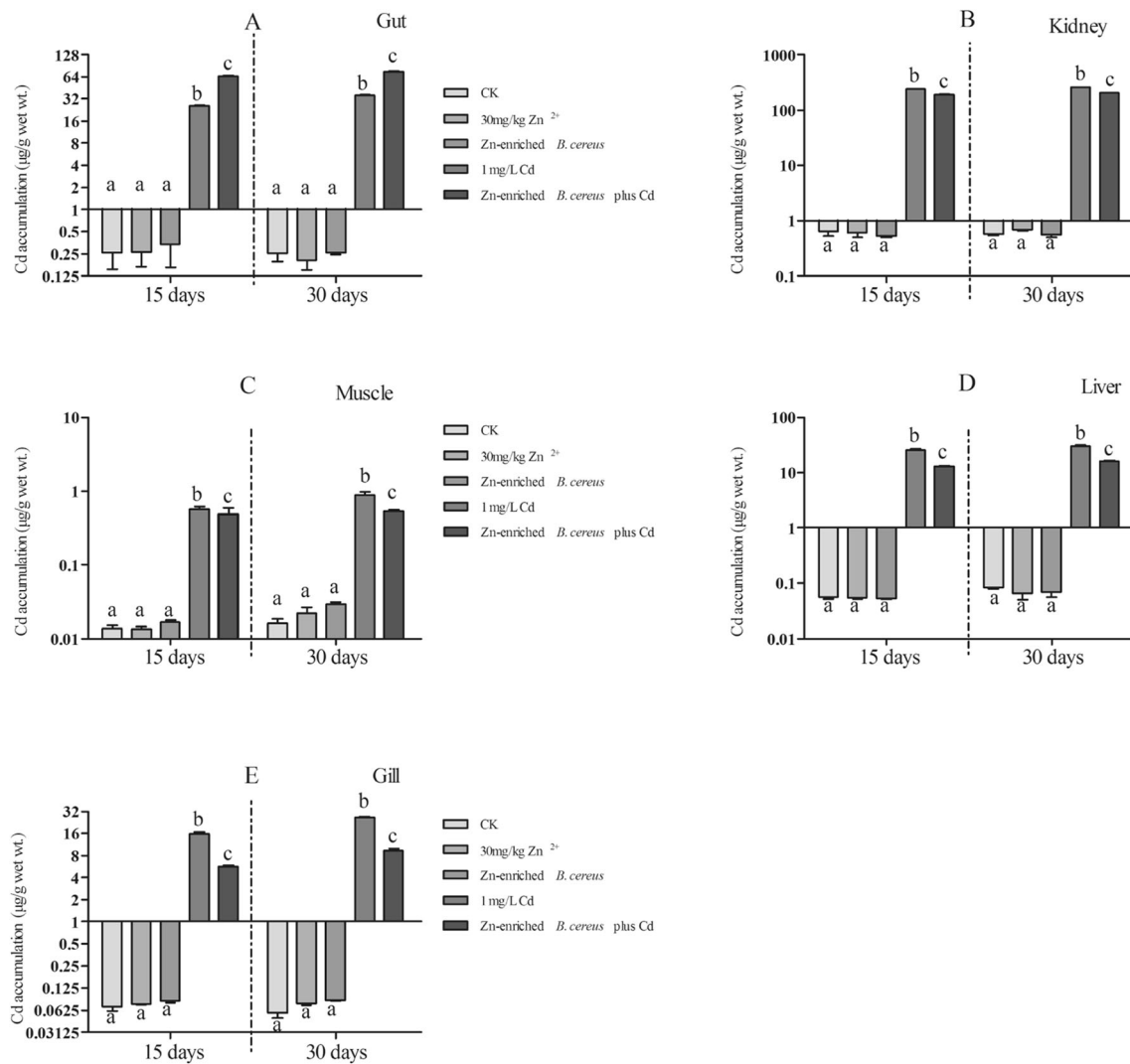
**Fig. 3** Cd accumulation (gut (A), muscle (B), liver (C), kidney (D), and gill (E)) of common carp ($n = 6$). Data are expressed as the mean ± SD. Bars with different letters are significantly different ($P < 0.05$) by Tukey's test on the same sampling interval

studied next. A few reports suggested that the pathogenic bacteria in fish were significantly increased in cadmium exposure [5, 10, 11]. A few authors suggested that *Flavobacterium* and *Pseudomonas* have been mainly isolated from fish, suggesting that they could be saprophytic or commensal organisms that may act as opportunistic fish pathogens [37, 38]. In this study, our data suggested that *Flavobacterium* and *Pseudomonas* were significantly increased in 1-mg/L Cd group. However, *Flavobacterium* and *Pseudomonas* were significantly increased in Zn-enriched *B. cereus*. Many reports suggested that reduced proportion of pathogenic bacteria can effectively alleviate intestinal inflammation [39, 40]. *Cetobacterium* is the most dominant microbiota in the intestine of fish, and this bacterium has been proven to produce vitamin B12 [41, 42]. Our results demonstrated that the abundance of *Cetobacterium* was reduced in 1-mg/L Cd groups. In previous studies, we observed the same result. Therefore, the increased abundance of these bacteria may enhance cadmium isolation

in the gut due to cellular accumulation and biological clearance mechanisms.

It is reported that the concentration of Cd can be increased in the tissues of aquatic animals through accumulation [4]. The bioaccumulation pattern of waterborne Cd in different tissues of *Mugil cephalus* is liver > gill > muscle > alimentary canal > heart [43]. Wang et al. demonstrated that the order of distribution of Cd in the organs was intestine > kidney > liver > gill > muscle [32]. Uptake routes (whether waterborne or diet-borne) of toxic substances affect tissue accumulation of toxicants in aquatic animals [44]. Liu et al. (2015) suggested that the profile of Cd accumulation in the organs of juvenile cobia was kidneys > liver > gut > gills [45]. In this study, the bioaccumulation pattern of waterborne Cd in different tissues was kidney > gut > liver > muscle > gill. In previous study, we reported that the *B. cereus* can reduce the Cd accumulation in *Carassius auratus gibelio* [32]. In addition, a few studies reported that dietary Zn can reduce the Cd accumulation in
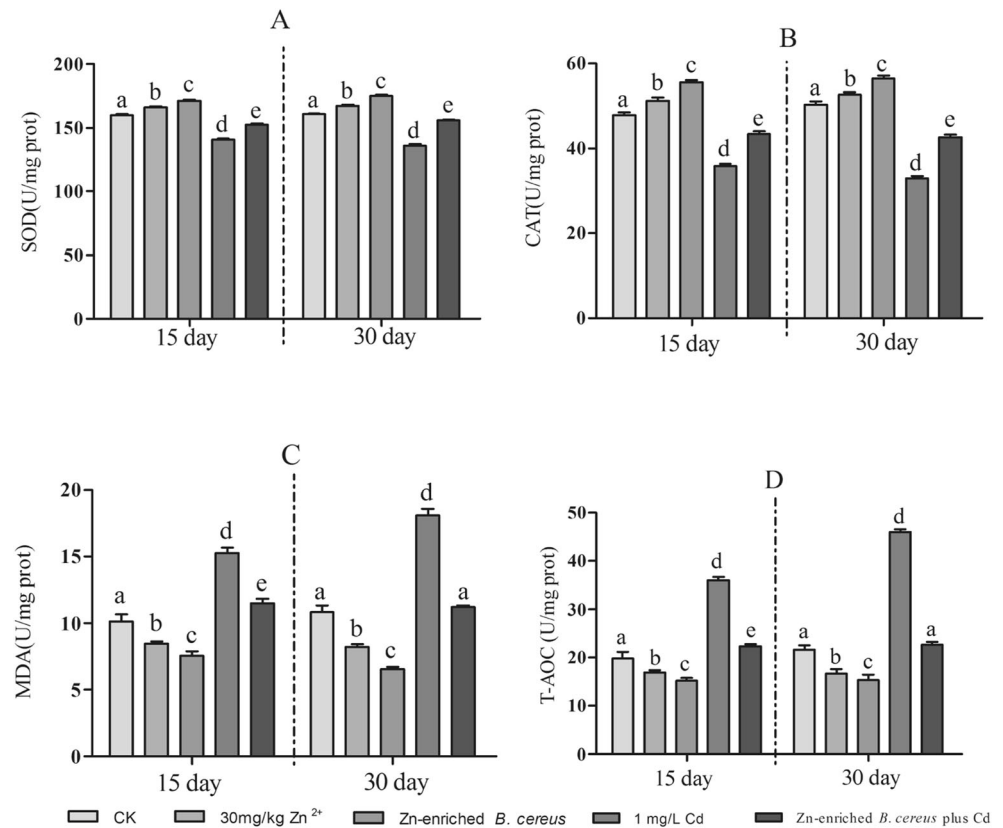
fathead minnow (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*) [17, 18]. Our results suggested that *B. cereus* shows the strongest for Zn biosorbed in vitro. In addition, we suggested that the Zn-enriched *B. cereus* can reduce the Cd accumulation in the organ of mirror carp. Many reports suggested that the accumulation of Cd can induce overproduction of reactive oxygen species (ROS) in many organisms, and this overproduction results in oxidative stress [34]. Wu et al. (2019) demonstrated that oxidative stress was significantly increased following waterborne Cd (299.6 μg/L) in female rare minnow (*Gobiocypris rarus*) [46]. Antioxidant enzymes, including SOD, CAT, and T-AOC, are the primary line of defense against ROS. Wang et al. (2020) reported that Cd can decrease SOD and CAT activity in human gastric epithelial cells [47]. A parallel study in grass carp kidney cells [48]. MDA, a product of lipid peroxidation, is a major contributor to the loss of cell function under oxidative stress. A few studies indicated that increased lipid peroxide levels (MDA levels) after Cd administration were previously demonstrated in rats [49]. In addition, Zhai et al (2014) reported that *Lactobacillus plantarum* CCFM8610 alleviates Cd toxicity in mice by oxidative stress (enhance SOD and CAT activity) and tissue damage [21]. In this study, our data suggested that SOD and CAT were significantly decreased in 1-mg/L Cd group and MDA and T-AOC were significantly increased in 1 m-/L Cd. In addition, dietary Zn and dietary Zn-

enriched *B. cereus* were significantly alleviated the oxidative stress in mirror carp. This finding is supported by the observation that Zn or *B. cereus* can enhance animal antioxidant activity. The accumulation of Cd in the organ of fish fed the Zn-nonenriched and Zn-enriched *B. cereus* was the likely causation of the localized oxidative stress. The antioxidative ability might be one of the main mechanisms for Zn-enriched *B. cereus* to alleviate Cd toxicity which needs to be further confirmed.

## Conclusion

In conclusion, our results suggested that Zn-enriched *B. cereus* can modulate intestinal microbiota, reduce Cd accumulation in organ, and change antioxidant activity following Cd exposure in mirror carp. These results indicated that Zn-enriched *B. cereus* may be useful as supplement to minimize the toxic side effects of cadmium in aquaculture.

## Declarations

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