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Selenium-enriched *Bacillus subtilis* reduces the effects of mercury-induced on inflammation and intestinal microbes in carp (*Cyprinus carpio var. specularis*)

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Abstract Mercury (Hg) is a global pollutant that affects the health of humans and ecosystems. Selenium (Se) is an essential trace element for many organisms including humans. *Bacillus subtilis* is one of the main probiotics used in aquaculture, and has a certain adsorption effect on heavy metals. The interaction between Hg and Se was rigorously studied, especially due to the observation of the protective effect of Se on Hg toxicity. The objective of this study was to research the effects of Hg, Se, and *B. subtilis* on inflammation and intestinal microbes in common carp. The common carp was exposed to Hg (0.03 mg/L), and 10^5 cfu/g Se-rich *B. subtilis* was added to the feed. After 30 days of feeding, samples were taken to evaluate the growth performance, serological response, inflammatory response, and intestinal microbial changes. In this study, when fish were exposed to Hg, the growth performance of the Se-rich *B. subtilis* plus 0.03 mg/L Hg fish group was lower than that of the control group and higher than 0.03 mg/L Hg; The levels of serum immunoglobulin M (IgM) and lysozyme (LZM) decreased, but after supplementation with Se-rich *B. subtilis*, the levels of LZM and IgM increased; Hg treatment significantly upregulated the mRNA expression of interleukin-1β (IL-1β), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and nuclear factor-kB (NF-κB P65), but downregulated the mRNA expression of interleukin-10 (IL-10), transforming growth factor-β (TGF-β) and NF-kappa-B inhibitor alpha (IkBα). However, compared with the Hg group, the Se-rich *B. subtilis* plus Hg group can significantly increase the mRNA expression levels of IL-1β, IL-8, TNF-α, and NF-κB P65, but reduce the regulation of IL-10, TGF-β, and IkBα expression. Through the analysis of the microbiological, we found that the Hg group was mainly composed of *Aeromonas sobria* and *Aeromonas hydrophila*. However, in the Se-rich *B. subtilis* treatment group, we found that *Aeromonas sobria* was significantly less than the Hg group. Se-rich *B. subtilis* improves Hg-induced intestinal microbial changes, alleviates the abundance of *Aeromonas*, and alleviates

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the inflammation of the fish. The results of this study show that Se-rich *B. subtilis* dietary supplements can effectively protect common carp against Hg toxicity.

Keywords *Bacillus subtilis* · Mercury · Selenium · Inflammation · Gut microbes

Introduction

Heavy metals have become serious pollutants in the aquatic environment due to their persistence to the environment and the ability to be accumulated by aquatic organisms (Veena et al. 1997). Mercury (Hg) is a global pollutant that has been associated with kidney immune and genetic damage to animals and humans, as well as microbial diversity and function (Liu et al. 2018a, 2018b). Exposure to Hg can cause various diseases of the organ system (Rice et al. 2014). Fish are exposed to Hg due to pollution in inland waters, which will lead to deterioration of fish health, thereby reducing fish quality and fish production (Begam and Sengupta. 2015). The pro-inflammatory transcription factor NF-kB p65 is often a central mediator of the immune and inflammatory response; Studies have found that mercury can significantly induce the upregulation of the pro-inflammatory transcription factor NF-kB p65 (Adegoke et al. 2018; Alexandrov et al. 2018). In the immune system, pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), are functionally active regulators of the innate immune response (Khansari et al. 2017; Sareila et al. 2011).

For a long time, it has been observed that Se protects animals from the toxicity of inorganic mercury and methylmercury. Parízek and Ostádalová reported one of the earliest studies on the protective effect of Se. This experiment Se protects rats from inorganic mercury-induced kidney poisoning (Parízek and Ostádalová 1976). Subsequent studies found that this is the absorption and interaction of mercury and Se in *Pseudomonas fluorescens* to achieve the detoxification of Se and mercury (Belzile et al. 2006).

Se is an essential micronutrient element that has a variety of complex effects on human health. Se is essential to human life and health, which is mainly due to its antioxidant, anti-inflammatory, and antiviral properties (Wrobel et al. 2016). Lin et al. reported that Se deficiency can reduce the growth performance of the kidney, spleen, and skin of the young grass carp head and impair its immune function (Zheng et al. 2018). At the same time, Se supplementation can alleviate the upregulation of nuclear factor NF-kB induced by microcystin-leucine arginine, and the upregulation of inflammatory cytokines IL-6, TNF-α, IL-1β, and TGF-β1 in cells (Adegoke et al. 2018).

Probiotics are living microorganisms that provide health benefits to the host when supplied in sufficient volumes (W.H. Organization 2001). According to many recent studies, probiotics derived from the host's intestinal tract increase the growth rate of the host by hydrolyzing the complex polysaccharides in the host's nutrients. As a live microbial feed supplement, it is beneficial to the development of the host. Probiotics such as microbes and viral dysentery can prevent the host from suffering from various diseases (Doeschate and Coyne 2008; Teemu et al. 2008; Zhao et al. 2012). In addition, compared to control group, the fish fed BS8 and LL8 showed higher gene expression levels of interleukin (IL-1β), interferon-γ (IFN-γ), heat shock protein 70 (HSP70), and tumor necrosis element (TNF-α) (Won et al. 2020). *B. subtilis* can upregulate vascular endothelial development element (VEGF) and hypoxia-inducible element-1α (HIF-1α) and influence the expression of mitogen-activated protein kinase (MAPK) and nuclear factor-kB (NF-kB) signaling pathways accelerate cell migration, regulate the secretion of pro-inflammatory cytokines, and the phenotypic transition of macrophages (Yan et al. 2020).

Previous reports paid more attention to the impact of heavy metals. However, there are few literature reports on the effects of probiotics and trace elements on the toxicity of heavy metals in aquatic animals. It is apparent that no attempt has been made so far to investigate the effect of Hg and/or Se-rich *B. subtilis* on the intestinal microbes of fish. Therefore, the objective of this study was to research the effects of Hg, Se, and *B. subtilis* on growth performance, inflammation, and intestinal microbes in common carp.

Materials and methods

Preparing Se-rich *B. subtilis* and preparing diet.

Commercial feed was a basic diet. Se-rich *B. subtilis* is added to the basic feed for the detailed steps of

preparing Se-enriched *B. subtilis* (please refer to previous research; Shang et al. 2021). The mercury content in 20-ml water samples from different aquariums was collected. Table 1 displays the actual Hg concentration. The probiotics are diluted with sterile normal saline, fully homogenized, and added to the basic feed according to the needs of the experiment (final dose of bacteria 10^5 cfu/g feed; final concentration of Se 0.5 ppm) (Shang et al. 2021). The same volume of sterile saline was added to the basic diet to prepare a control. Store all feed in a refrigerator at 4℃.

Feed and experimental design

Common carp (*Cyprinus carpio var. specularis*; 6.2 ± 0.1 g) was purchased from an aquatic fry farm (Jilin Province, China) and transported to the laboratory. We randomly divided 360 fish into four groups and divided these groups evenly into 12 tanks (80 l; 3 replicates per group; 30 fish per tank). The common carps were cultured at 24 ± 2 °C in recirculating tanks for 2 weeks to adaptive the environment. During this time, they were fed twice a day at 8 o'clock and 18 o'clock. The healthy common carps were randomly divided into 4 treatment groups (Se-rich *B. subtilis*, control group, Se-rich *B. subtilis* plus 0.03 mg/L Hg, and 0.03 mg/L Hg) (Zhang et al. 2016a). After the experiment started, they were fed twice a day at 8 o'clock and 18 o'clock for 30 days, according to the fish body mass accounting for 1–2% of the daily feeding amount (Zhang et al. 2016a). In 80 l aerated tap water in the water tank, the daily water exchange rate is 1/2 of the total. Then, six fish blood samples, spleen, kidney, and intestine tissues were randomly collected for follow-up experiments. The blood sample was collected from the caudal vein of the individual fish after they were anesthetized with benzocaine.

Table 1 Nominal and actual Hg concentration in water (mg/L)

Groups	Nominal concentration	Actual Hg concentration
control group	0	0.005 ± 0.001^{a}
Se-rich *B. subtilis*	0	0.003 ± 0.001^{a}
Se-rich *B. subtilis* plus 0.03 mg/L Hg	0.03	0.029 ± 0.002^{b}
0.03 mg/L Hg	0.03	0.005 ± 0.001^{b}

Note: The actual Hg concentration in water at 30 ($n = 12$) days for each group. Data are mean ± S.D. Bar with different letters are significantly ($P < 0.05$) on the same sampling interval

Growth performance

Observe the development performance of common carp after one month of breeding (Chen et al. 2017). The calculation of coefficients was made below: rate of survival (SR, %) = 100 × (ultimate quantity of fish/initial amount of fish), weight gain proportion (WGR, %) = 100 × [(ultimate body weight − primary body weight)/primary body weight], given rate of increase (SGR, %/day) = 100 × [(ln ultimate body weight – ln primary body weight)/days].

Serum immunological test

Elisa kit (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, Jiangsu) is used to determine serum immunoglobulin M (IgM) levels and lysozyme (LZM) activity.

Reverse-transcriptase real-time PCR

At the end of the exposure test, the expression levels of immune-related genes in the spleen and kidney tissues were measured. The Trizol tool (Takara, Dalian, China) was used to extract total RNA from the spleen and kidney. Use RT-PCR cDNA tool (Takara, Dalian, China) to synthesize clean RNA with OD260/OD280 absorption ratio 1.8–2.0 as a template (Wang et al. 2019). The primers were synthesized by Kumei Biotechnology Co., Ltd., Jilin. RT-PCR is used to quantify the expression levels of 7 immune response-related genes (IL-8, NF-kB P65, IkBα, IL-1β, TNF-α, IL-10, and TGF-β). Use housekeeping gene β-actin as an internal control (Yin et al. 2018). Table 2 shows the sequence of the given primers used in this study. The RT-PCR reaction takes a total volume of 20 ul, including 1 ul cDNA, 2 ul each primer, 7 ul treated DEPC water, and 10 ul SYBR Premix Ex Taq Master Mix. The thermal reaction conditions are as follows: 95 °C for 5 min, 95 °C for 5 s, 60 °C for 30 s, 72 °C for 30 s, cycle 30 times. The RT-PCR reaction is repeated 3 times for each sample. Convert the data to Ct values after each reaction. The relative gene expression is determined by $2^{-\Delta\Delta CT}$.

Table 2 Primers used in this study

Genes	Sequences (5′-3′)		Accession no
β-actin	Forward	TGAAGATCCTGACCGAGCGT	NM_131031.1
	Reverse	GGAAGAAGAGGCAGCGGTTC	
IL-1β	Forward	ACCAGCTGGATTTGTCAGAAG	AB010701
	Reverse	ACATACTGAATTGAACTTTG	
IL-8	Forward	ATGAGTCTTAGAGGTCTGGGT	JN663841
	Reverse	ACAGTGAGGGCTAGGAGGG	
TNF-α	Forward	GGTGATGGTGTCGAGGAGGAA	AJ311800
	Reverse	TGTCATCCTTTCTGCTCTGGTT	
IL-10	Forward	GGAAAGACACCTGGCTGTA	JX524550.1
	Reverse	CCACAAATGAGCAACAGTCA	
NF-kB p65	Forward	GGCAGGTGGCGATAGTGTT	AY735398.1
	Reverse	CATTCCTTCAGTTCTCTTGCG	
IkBα	Forward	TCTTGCCATTATTCACGAGG	KJ125069
	Reverse	TGTTACCACAGTCATCCACCA	
TGF-β1	Forward	TTGGGACTTGTGCTCTAT	EU099588
	Reverse	AGTTCTGCTGGGATGTTT	

DNA extraction and 16S rRNA gene exploration

On the last day of mercury exposure, 3 fecal samples were randomly selected from the control group and the treatment group, immediately frozen in liquid nitrogen, and stored at − 80 °C. The QIAamp DNA Stool Mini Tool (Qiagen, Germany) was used to extract microbial DNA from the carp face. PCR amplification uses 16S variable region V3–V4 universal primers. Use the Quantitative Analysis of Microbial Ecology (QIIME) tool (version 1.17) to analyze the raw readings. UPARSE is used to cluster OTU, with an analogy cutoff rate of 97%, and UCHIME is used to identify and remove chimeric sequences. Using the RDP classifier against the SILVA (SSU115) 16S rRNA database, with a confidence threshold of 70%, it is used to analyze the classification of each 16S rRNA gene sequence.

Statistical exploration

SPSS 20.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Information was shown as mean ± standard deviation (S.D.) for every group. The whole test was made for three times. One-way exploration of variance (ANOVA) was adopted for the determination of the significance variations among the groups, which was followed by Tukey's various contrast experiment. The significance level was set at $P < 0.05$.

Results

Se concentration measurement on Se-rich *B. subtilis*, and common carp growth performance.

The plate counting approach was used to test the tolerance of *B. subtilis* for Se. A Se concentration of 0.5 g/l was used in the current test (the transformation rate of Se is 56.2%) (Shang et al. 2021). The growth performance of common carp is shown in Table 3. The survival rate of common carp was 100%, and there was no significant difference. With the increase of dietary Se-enriched *B. subtilis* levels, WGR and SGR showed the same trend. There was no significant difference between the control group and the Se-enriched *B. subtilis* group, while the 0.03 mg/l Hg group was significantly reduced compared to the control group ($P < 0.05$). The growth performance of the se-rich *B. subtilis* plus Hg fish group of 0.03 mg/l was less than that of the control group and more than 0.03 mg/l Hg ($P < 0.05$).

Table 3 Growth performance of *C. carpio var. specularis* fed the experimental diets for a month

	Diet			
	Control group	Se-rich *B. subtilis*	Se-rich *B. subtilis* plus 0.03 mg/L Hg	0.03 mg/L Hg
Initial BW (g)	6.25 ± 0.13	6.25 ± 0.14	6.27 ± 0.13	6.27 ± 0.14
Final BW (g)	14.17 ± 0.12^{c}	14.27 ± 0.34^{c}	13.12 ± 0.01^{b}	12.27 ± 0.06^{a}
WGR (%)	128.32 ± 1.95^{c}	126.32 ± 5.50^{c}	109.97 ± 0.24^{b}	96.37 ± 1.08^{a}
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
SGR (%/day)	26.40 ± 0.40^{c}	26.73 ± 1.14^{c}	22.91 ± 0.05^{b}	20.08 ± 0.22^{a}

Data represent mean ± S.E.M of three replicates

Note: Mean values within a row unlike superscript letters were significantly different ($P < 0.05$)

Initial BW, initial body weight; *Final BW*, final body weight; *WGR*, weight gain ratio; *SR*, survival rate; *SGR*, specific growth rate

Serum non-specific immune responses

Hg is known to cause disturbances in the immune response. LZM and IgM levels for both treatment and control groups were determined (Fig. 1). When fish were exposed to Hg, LZM and IgM levels decreased. However, LZM and IgM levels increased after supplementation with Se-rich *B. subtilis*. The LZM and IgM extents of the Se-rich *B. subtilis* group grew greatly by comparing with the control group ($P < 0.05$; Fig. 1).

Immune-associated gene expression

Hg exposure greatly upregulated the mRNA expression of IL-8, IL-1β, TNF-α, and NF-kB P65 but downregulated the mRNA expression of IL-10, TGF-β, and IkBα (Fig. 2 A, B, C, D). Nevertheless, the co-treatment with Hg and Se-enriched *B. subtilis* greatly increased the mRNA expression levels of IL-8, IL-1β, NF-kB P65, and TNF-α. Compared with the group exposed to Hg and not supplemented with dietary supplements, downregulate the mRNA expression of IL-10, TGF-β, and IkBα (Fig. 2 A, B, C, D) ($P < 0.05$). Compared with the control group, IL-1β, TNF-α, IL-8, and NF-kB P65 were upregulated by exposure to Hg. The consumption of Se-rich

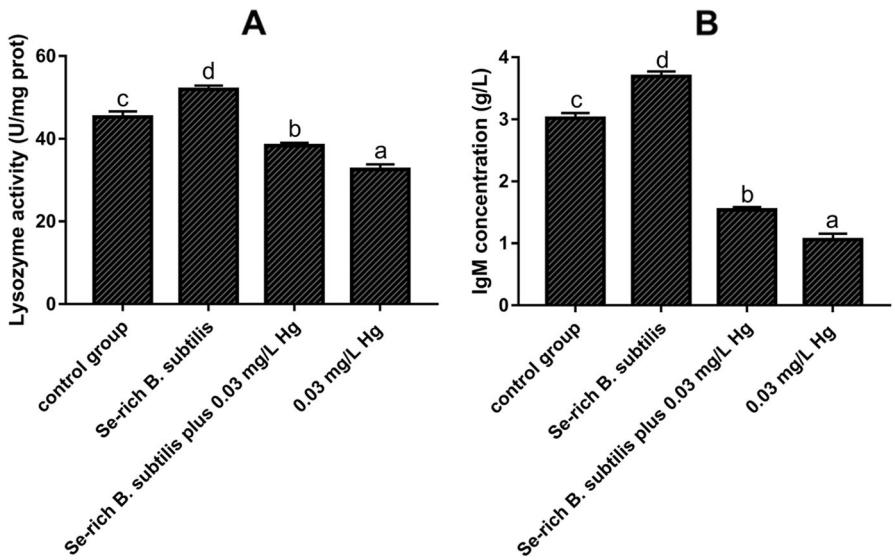

Fig. 1 Effects of selenium-rich *Bacillus subtilis* and/or Hg on LZM and IgM activity levels in the blood of common carp ($n = 6$). Values are presented as means ± S.D. and indicate significant differences ($P < 0.05$) between treated groups and the control group

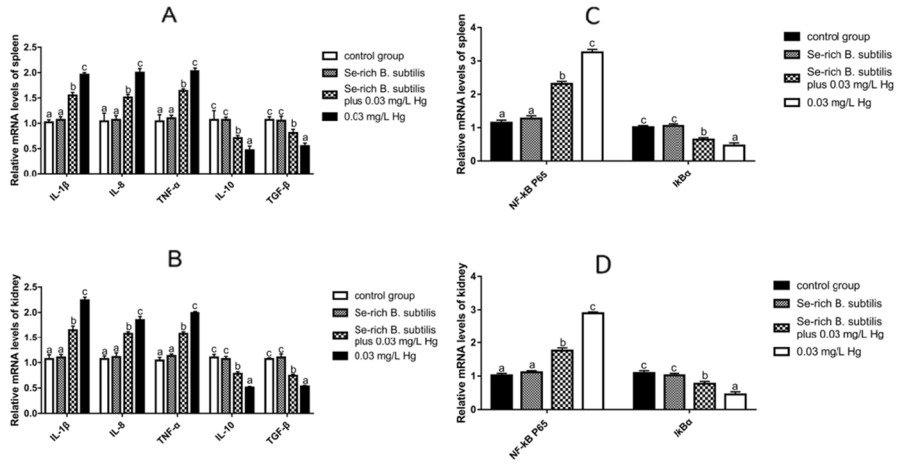

Fig. 2 Effects of selenium-rich *B. subtilis* and/or Hg on the mRNA level of IL-1β, IL-8, TNF-α, IL-10, and TGF-β in the spleen (A) and kidney (B) of common carp ($n=6$). Effects of selenium-rich *B. subtilis* and/or hg on the mRNA level of NF-kB P65 and IkBα in the spleen (C) and kidney (D). Each value is presented as the mean ± S.D. and indicates significant differences between the treated groups and the control group ($P<0.05$)

B. subtilis alleviated IL-1β, TNF-α, IL-8, and NF-kB P65 were upregulated, and IL-10, TGF-β, and IkBα were downregulated ($P<0.05$).

DNA extraction and 16S rRNA gene exploration

Statistical exploration of sequencing data

The dilution curve directly shows the rationality of the amount of sequencing data and indirectly shows the abundance of species in the sample. If the curve tends to be flat, it indicates that the amount of sequencing data is gradually reasonable. In this study, after a month of feeding trials, we found that the end of the thinning curve (Fig. 3A) was flattened. Therefore, we conclude that the amount of sequencing data is reasonable for our analysis.

For clarifying the effect of Hg in the intestinal flora of common carp, we performed PCoA analysis. The control group, the Se-rich *B. subtilis* group, the Se-rich *B. subtilis* plus Hg group, and the Hg group were combined and analyzed. The PCoA results showed that the microbial composition of the four groups of different diets was significantly different (Fig. 3 B) ($P<0.05$).

The chao1 index (the number of species included in the community) between the four groups found that the control group was relatively high, but the difference was not significant (Fig. 3 C). The Shannon index (the diversity of gut microbes) found that there was no significant difference between different diets and groups (Fig. 3 D). The results showed that the species richness and uniformity of each group of different diets did not change much.

Comparison at the genus levels

All sequences were identified at the genus level. We selected thirty data from the genus for analysis. The five main genera in the control group were *Verrucomicrobiaceae*, *Cetobacterium*, *Pseudorhodobacter*, *Gemmobacter*, and *Aeromonas*. The most common genera in the Hg group include *Verrucomicrobiaceae*, *Gemmobacter*, *Cetobacterium*, *Aeromonas*, and *Pseudomonas*. After Hg exposure, the abundance of *Aeromonas* and *Roseomonas* increased significantly ($P<0.05$). At the same time, after Hg exposure, the abundance of *Pseudorhodobacter* and *Verrucomicrobiaceae* was significantly reduced ($P<0.05$). However, in the Hg treatment group, we found that the increase of *Aeromonas* and *Rosemonas* was reduced. The decrease of *Pseudomonas* and *Verrucomicrobiaceae* was suppressed ($P<0.05$). In addition, we also found that *Verrucomicrobiaceae* in the Se-rich *B. subtilis* group also significantly decreased (Fig. 4 A, B) ($P<0.05$).

Comparison at the species level

Similarly, we selected 30 data from the species for analysis. The most important species of intestinal microbes in the Hg group are *Verrucomicrobiaceae_unclassifie*, *Aeromonas_sobria*, *Aeromonas hydrophila*, and *Aeromonas* spp. The most microbes in the control group were *Cetobacterium_somerae*,

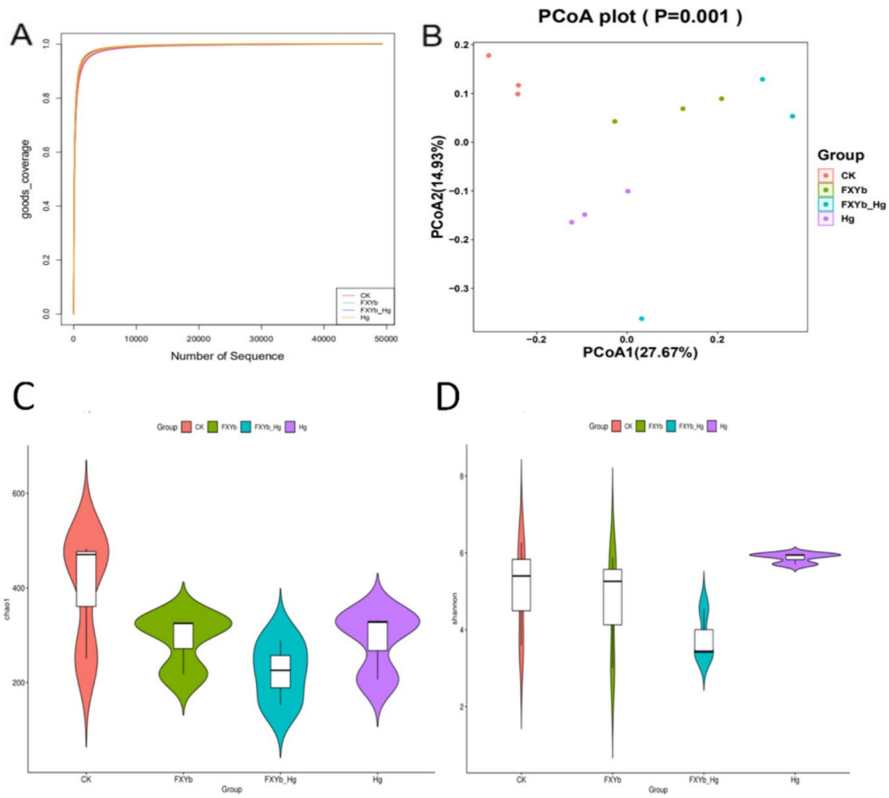

Fig. 3 **A** Rarefaction curves and estimators of the different samples. **B** Constrained PCoA plot of Bray–Curtis distances between samples by different dietary lipid ($P < 0.05$); alpha diversity of CK to FXYb, CK to FXYb + Hg, and CK to Hg (**C** and **D**)

Gemmobacter_sp._yp3, and *Pseudomonas_poae*. Compared with the control group, after Hg exposure, the abundance of *Cetobacterium_somerae*, *Pseudomonas_poae*, *Verrucomicrobiaceae_unclassified*, and *Gemmobacter_sp._yp3* were significantly reduced, and *Aeromonas sobria*, *Aeromonas hydrophila*, and *Aeromonas hydrophila* were significantly increased ($P < 0.05$). At the same time, in the Hg treatment group, it was found that the increase of *Aeromonas sobria*, *Aeromonas hydrophila*, and *Aeromonas* spp. was suppressed, while the decrease of *Verrucomicrobiaceae_unclassifie* was suppressed. *Pseudomonas_poae* and *Cetobacterium_somerae* increased significantly (Fig. 4 C, D) ($P < 0.05$).

Discussion

Probiotics improve animal health and nutrition by improving feed value and enzymatic effects, and play a very important role in improving animal health, nutrition, and activating immune response (Dawood et al. 2016). Zaineldin et al. reported that that supplementation of *Bacillus subtilis* in the diet can significantly improve growth performance (FBW, WG, and SGR) (Zaineldin et al. 2018). Hg can activate energy-consuming detoxification processes, which consumes a lot of energy in the fish, resulting in a decrease in energy, which is not conducive to the growth of the fish (Sfakianakis et al. 2015). In this study, although the Se-rich *B. subtilis* group did not show an increase in WGR and SGR compared to the control group, it was found to alleviate the growth performance of common carp affected by Hg.

Detection of autoantibodies may detect damage after metal exposure (El-Fawal et al. 1999). Studies have found that exposure to Hg changes and increases IgM levels (Osuna et al. 2014; Queiroz et al. 1994) However, in this study, we observed a decrease in IgM and LZM levels, which may be due to excessive accumulation of Hg in the blood. However, compared with the Hg group Hg plus Se-enriched *B. subtilis* group, the levels of IgM and LZM increased significantly. It has been shown that dietary supplementation with nano-Se significantly increased IgM levels and enhanced immune function in chickens (Cai et al.

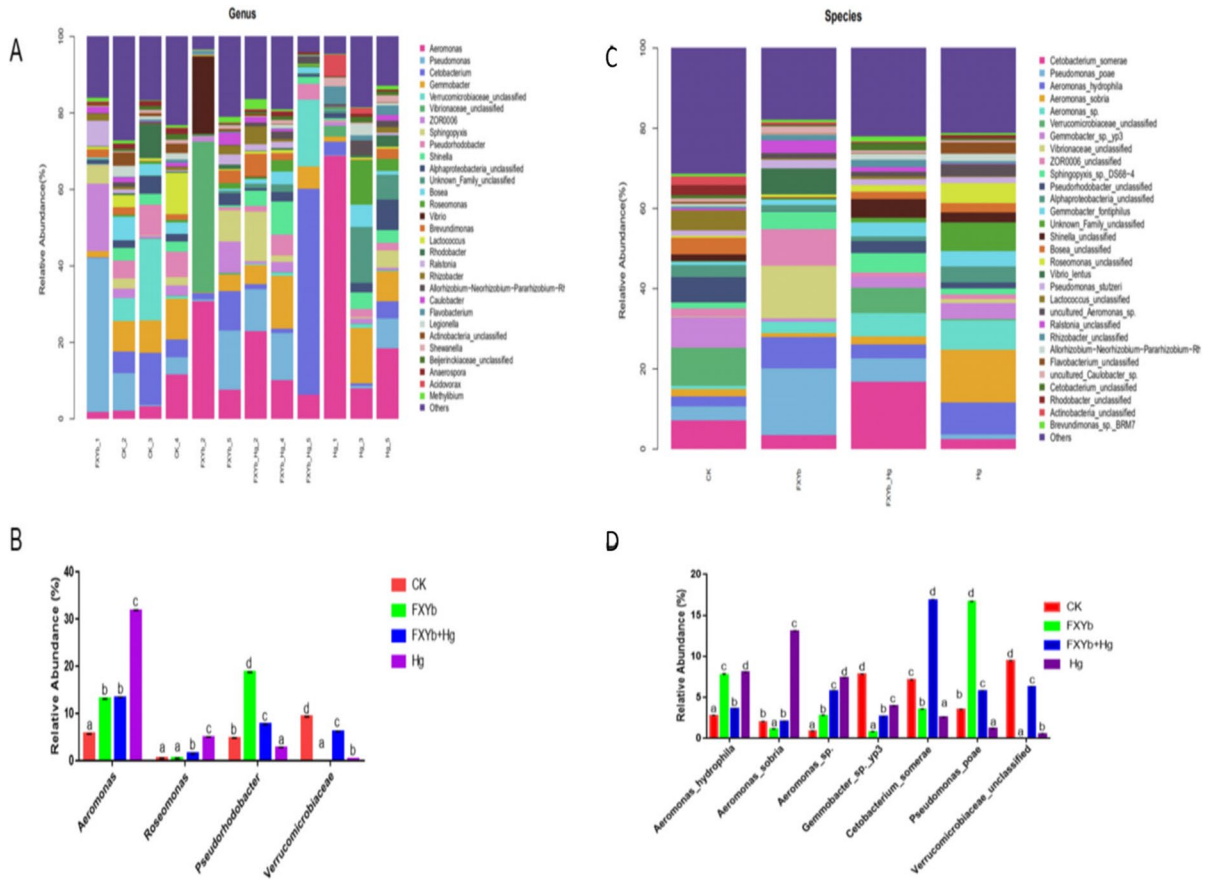

Fig. 4 (A) Relative abundance of the top 30 genus in the fecal microbiota between healthy and Hg-treated fish. (B) *t*-test analysis of different genus in fecal microbiota at the genus level; (C) relative abundance of the top 30 species in the fecal microbiota between healthy and Hg-treated fish. (D) *t*-test analysis of different species in fecal microbiota at the species level $P < 0.05$ indicates a significant difference between the four groups

2012; Kumar et al. 2012). In addition, it has been reported that the addition of *Bacillus licheniformis* to the carp diet increased LZM levels and enhanced disease resistance (Kumar et al. 2012). The secondary metabolites of probiotics may have beneficial effects on the host (Dennis-Wall et al. 2017). Secondary metabolites from probiotics are transported to host organs via blood circulation (Eloe-Fadrosh et al. 2015). Environmental pollutants such as heavy metals can affect the body's immune system and cause a decline in immunity. Cytokines, including, TNFs, and chemokines (Hawley et al. 2009; Savan and Sakai 2006). IL-1β, IL-8, and TNF-α are crucial pro-inflammatory cytokines that regulate the inflammatory process and are considered good markers of the inflammatory response (Chen et al. 2017). IL-1β mainly regulates the body's immunity against pathogens, and it regulates the activation of immune cells and non-immune cells in infected sites and organs; IL-8 promotes the migration of neutrophils to fight pathogens and can increase the expression of other cytokines (Tomalka and Hise 2015; Zhang et al. 2012). TNF-α is a multifunctional inflammatory cytokine, which can induce a variety of responses, including cell proliferation and apoptosis (Arnett et al. 2001). Cytokines can be regulated by nuclear factor kappa B (NF-κB) signaling. In the present study, Hg exposure upregulated IL-1β, IL-8, and TNF-α mRNA expression in the liver and spleen, consistent with results in zebrafish larvae (Zhang et al. 2016b). This indicates that Hg exposure triggered an inflammatory response. The changes in the expression of

these genes may be due to the accumulation of Hg in 30 days, which triggers a pro-inflammatory immune response and upregulate IL-1β, IL-8, and TNF-α. However, treatment with Se-rich *B. subtilis* attenuated the upregulation of IL-1β, IL-8, and TNF-α. Studies have shown that Se can upregulate chicken immune cytokines (i.e., IL-10 gene) (Xu et al. 2015). These results may indicate that anti-inflammatory cytokines effectively suppressed the pro-inflammatory immune response, which is consistent with the upregulation of IL-10 observed in this study. In addition, the upregulation of IL-10 in the liver may represent an aspect of the homeostatic mechanism that controls the Hg-induced inflammatory response. Gao et al. reported that the reduction of TGF-β will aggravate the inflammatory damage of liver tissue, but the lack of Se will inhibit the expression of TGF-β and promote the production of TNF-α, IL-1β, and IL-6, which may cause carp liver tissue inflammation, but Se supplementation can prevent the decrease of TGF-β (Gao et al. 2019). The intake of Se-rich *B. subtilis* will not only increase the Se content in the body, but also *B. subtilis* will absorb Hg and alleviate the damage of the fish (Shang et al. 2021). In this study, there may be such a mechanism. Hg intake reduced the expression of TGF-β, while the Se-rich *B. subtilis* plus Hg group alleviated the decrease of TGF-β. The transcription factor NF-κB controls the expression of inflammatory cytokine genes (Taro and Shizuo 2007). It controls the expression of pro-inflammatory genes and is also a key target for regulating inflammatory diseases (Xu et al. 2005; Yang et al. 2007). Study demonstrated that by catalyzing the degradation of IkBα, NF-κB can be activated by IKK (including IKKα, IKKβ, and IKKγ), which plays an important role in regulating human pro-inflammatory cytokines (Jobin and Sartor 2000; Bollrath and Greten 2009). In this study, we found that the expression of IkBα in the liver and spleen decreased, and the corresponding NF-κB p65 expression increased, and this phenomenon was alleviated in the Se-rich *B. subtilis* treatment group. So, there may be such a mechanism, and Se-rich *B. subtilis* may be involved in the regulation of the IkBα/NF-κB signaling pathway. When the body consumes too much Hg, it leads to insufficient Se content in the body and triggers the inflammatory response and activates the IkBα/NF-κB signaling pathway. After feeding Se-rich *B. subtilis* to supplement Se, Se inhibits the upregulation of pro-inflammatory cytokines in the cells and promotes the expression of anti-inflammatory cytokines, thereby reducing the harm of Hg to the fish.

The intestine is a complex ecosystem, and the intestinal flora has an important role in this ecosystem. Intestinal flora can assist the digestion and absorption of food and promote nutrient metabolism (Sommer and Backhed 2013). Changes in the intestinal flora can lead to disorders of the body's normal physiological functions, leading to diseases (Nicholson et al. 2012). Through previous studies, we found that Hg significantly reduced the activity of enzymes such as CAT and GSH-PX and triggered inflammation (Shang et al. 2021). This experiment uses Illumina high-throughput sequencing technology to explain how the composition and diversity of carp intestinal microbial communities change under Hg exposure conditions, and provide a theoretical basis for fish intestinal health and normal human growth and development. In this study, the levels of *Aeromonas sobria* and *Aeromonas hydrophila* in the intestine of common carp after Hg treatment were higher than those in the control group. Many studies have shown that changes in the diversity of intestinal flora can cause diseases such as enteritis, inflammatory diseases, and obesity (Chassaing and Gewirtz 2014; Beaz-Hidalgo and Figueras 2013). Therefore, Hg-induced changes in intestinal flora may affect the health of common carp.

In this study, our results indicate that *Verrucomicrobiaceae*, *Cetobacterium*, *Pseudorhodobacter*, *Gemmobacter*, and *Aeromonas* are the most important bacterial groups in common carp. The main flora in the intestines after Hg exposure are *Verrucomicrobiaceae*, *Gemmobacter*, *Cetobacterium*, *Aeromonas*, and *Pseudomonas*. Hg exposure caused changes in the intestinal flora, and it was found that the abundance of *Aeromonas* in the Hg treatment group was much higher than that of the control group. *Aeromonas* can colonize and infect the host, and can cause diseases such as sepsis and fungal infections. The extracellular products (hemolysin, lipase, and protease) produced by *Aeromonas* can cause soft tissue, hepatobiliary system, respiratory system, and arthritis disease (Elorza et al. 2020; Lian et al. 2020). In this study, Hg exposure increased the proportion of *Aeromonas* in the intestines of fish. However, in the Se-rich *B. subtilis* plus Hg group, we found that the abundance of *Aeromonas* was reduced, which

indicates that feeding the Se-rich *B. subtilis* can change the intestinal microbes of the fish and reduce the abundance of *Aeromonas*. *Aeromonas sobria* can cause oxidation in fish bodies to change superoxide dismutase, glutathione peroxidase, and upregulate immunoglobulins IgM and TNF-α (Harikrishnan et al. 2020). *Aeromonas hydrophila* can cause *Catla catla* immune response and increase IL-1β and TNF-α (Harikrishnan et al. 2021). In this study, it was found that *Aeromonas sobria* and *Aeromonas hydrophila* were significantly increased, which may be another cause of the disease. Hg induction will change the *Aeromonas* in the common carp intestine, and increase the *Aeromonas sobria* and *Aeromonas hydrophila* in the *Aeromonas*, which leads to an inflammatory response in the fish. Se-rich *B. subtilis* through the action of Se and the probiotic *B. subtilis* regulates the IKBα/NF-κB signaling pathway and reduces the inflammatory response. The composition of the intestinal flora was detected by 16S rRNA gene sequencing, and this phenomenon may be that the Se-rich *B. subtilis* improved the intestinal flora and reduced the abundance of *Aeromonas*, thereby reducing the inflammatory response.

Conclusions

In conclusion, our results reported the effect of Se-rich *B. subtilis* on common carp exposed to mercury. This provides insightful insights for the Se-rich *B. subtilis* to reduce mercury poisoning in common carp. In this study, Se-rich *B. subtilis* alleviates mercury-induced effects on common carp growth performance and inflammation by changing the changes of intestinal microbes.

Author contribution The specific work of each author in this study was as follows: Xinchi Shang, perception and design; surgical operation; final approval of the version to be published; Bo Wang, participation in the whole work; drafting of the article; data analysis; Qingsong Sun: methodology. Yue Zhang, methodology, investigation, writing-original draft. Yuting Lu, investigation. Shaojun Liu, methodology. Yuehong Li, investigation, writing-original draft. Thank you and best regards.

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Declarations

Ethics approval All experimental and animal handling procedures were conducted according to the research protocols approved by the Institutional Animal Care and Use Committee, Jilin Agricultural University, Jilin Province, China.

Consent to participate The author of the article is approved by everyone.

Consent for publication All authors agree to publish this article to Fish Physiol Biochem.

Competing interests The authors declare no competing interests.

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