Antioxidant Activities of Pediococcus Acidilactici GR-66 and Proposed Chromium (VI)-reducing Mechanism

Yunyue Wang

Shenzhen College of International Education, Shenzhen 518040, China

Abstract. Chromium (Cr), one of the most common heavy metals in industry, is an environmental pollutant and has been recognized as a carcinogen to humans. Some probiotics can reduce oxidative stress induced by Cr (VI), but the underlying mechanism has not been fully uncovered. In this study, five probiotic strains have been identified from fermented milk of yak and assessed their antioxidant capability. *P.acidilactici* GR-66 demonstrates the greatest antioxidant capability in vitro and then was orally administered to mice for 3 weeks as the treatment to Cr(VI) exposure. Results manifest that GR-66 can significantly alleviate Cr (VI)-induced inflammation and oxidative stress in vivo. Since the colonization number of GR-66 is less than 1%, GR-66 was not directly involved in intestinal Cr (VI) remediation but adjusted the gut microbiota. Sequencing of GR-66 recognized gene comp998 encoded enzyme that has been later aligned with NCBI database and identified a structure similar to flavodoxin 2HAY. Thus, we proposed that the comp998 reduce Cr (VI) to Cr (III) by transferring electrons through FMN.

Keywords: Gut Remediation; Probiotics; Chromium (VI); Oxidative Stress.

1. Introduction

The increasing anthropogenic activities have aggravated heavy-metal contamination, which causes detrimental impacts on the human health and environment (Mitra et al., 2022; Shanker et al., 2005). Chromium (Cr) is an ubiquitous heavy metals applied in industries, such as metallurgy, tanning, electroplating, and chemical production (Barnhart, 1997). Due to high solubility, Cr released with untreated discharge can easily contaminate soil and water sources, eventually accumulating in human bodies (Chary et al., 2008; Oliveira, 2012). As a well-known carcinogen, Cr can induce a multitude of harmful effects, including ulceration, damage to the gastrointestinal system, epigenetic modulation, etc (Sun et al., 2015; Unceta et al., 2010). Although numerous technologies have been developed to remove heavy metals, Cr remediation is generally limited by the high cost like membrane filtration and long duration like phytoremediation (Madhavi et al., 2013). Some Cr-microorganisms have been identified and further adopted to remediate the contamination, showing the potential as an effective and efficient method to attenuate Cr-induced effects (Kamaludeen et al., 2003; Mishra et al., 2017).

Currently, it has been proven that gut remediation by probiotics can be a novel, secure and efficacious method to reduce the damage induced by biotoxicity. The mutualistic relationship between gut-microbial and human is an essential component of bacterial colonies in specific regions, conducing to hosts' health (FAO/WHO, 2001; Feng et al., 2018). So far, some probiotics have been reported to involve in Cr (VI) detoxification. For instance, species of *Lactobacillus*, and *Streptococcus* have a strong ability to bind and detoxify Cr(VI) (Younan et al., 2016). *Lactobacillus rhamnosus*, *Bacillus* and *Lactobacillus acidophilus* have a great Cr-reducing ability (Liu et al., 2006) (Upreti et al., 2011); Mouse model has demonstrated that *Lactobacillus casei* can alleviate the oxidative stress caused by excess intake of Cr(VI) (Balakrishnan et al., 2014). More specifically, oxidative stress has been identified as one of the main mechanisms of the Cr toxicity (Luo et al., 1996; Shi et al., 1994; Shi et al., 1992; Tsapakos & Wetterhahn, 1983).

Under the conditions of high altitude and strong radiation in the Qinghai-Tibet plateau, we hypothesize that the local bacteria have a remarkable antioxidant capability (Liu et al., 2022). Thus, bacterial strains isolated from the traditionally fermented milk of yak at the Tibetan plateau have their antioxidant capacity compared, among which *Pediococcus acidilactici* GR-66 has the greatest antioxidant capacity. The detoxification effects of GR-66 have then been determined by mouse

models to further investigate the level of intestinal colonization, alleviation of oxidative stress, effect on the Cr-mediation of transcriptome and mechanism for Cr-mediation.

2. Result

2.1 GR-66 has Great Antioxidant Abilities in Vitro.

First, the optimum growth conditions of five probiotics (*Pediococcus acidilactici* GR-66, *Lacticaseibacillus casel* LZ-2, *Lacticaseibacillus casel* XS2, *Lactobacillus acidophilus* XN-3, *Lactiplantibacillus plantarum* TT2) were determined. After 24-hour culture at 37°C with initial OD600 = 0.1, the results demonstrate that aerobic and stationary culture in MRS medium is the optimum culture condition, among which GR-66 shows the best performance of which OD600 = 3.92 (Fig. S1). Subsequently, these probiotics were exposed to 1 mM Cr (VI). After 4 h, GR-66 reduced nearly 30% of Cr (VI), while other strains reduced less than 20% of Cr (VI) (Fig 1A). Besides, after overnight exposure to 1 mM Cr (VI), tolerance to Cr (VI) of five strains was reflected by the OD600 value (Fig.1 B). OD600 of GR-66 was 1.15, while other strains were below 0.8. These results showed that GR-66 had the highest Cr (VI) reduction and tolerance.

Tolerance H_2O_2 of five probiotic strains were determined by measuring OD_{600} after overnight exposure to 1 mM H_2O_2 (Fig.1C). The OD_{600} of GR-66 was 1.14 after overnight exposure to H_2O_2 which was at least 0.4 higher than the OD600 of other strains. Accordingly, the hydroxyl group scavenging ability of five strains was tested and calculated into percentages (Fig.1 D). GR-66 was able to scavenge 72.5% of the hydroxyl group which was slighter greater than XN-3 and much greater than the remaining three. DPPH radical scavenging ability of five probiotic strains was measured because it indicates the ability to supply proton (Fig1 E). GR-66 was able to scavenge 54.4% of DPPH radical, which was slighter greater than XS2 and much greater than the others. The total antioxidant capacity of five probiotic strains was determined by measuring the concentration of Fe (II) reduced from Fe (III), amidst which GR-66 reduced about 2-fold Fe (III) than the other 4 strains with Fe (II) concentration of 0.31 mM/mL (Fig.1 E). The high Cr (VI) reducing ability, tolerance toward Cr (VI) and H2O2, hydroxyl group and DPPH racial scavenging ability, and total antioxidant capacity suggested the great potential of GR-66 to resist chromium stress and oxidative stress. Thus, GR-66 was chosen for the following experiments in mice.

2.2 *Pediococcus acidilactici* GR-66 Treatment Alleviates the Gr(VI)-induced Weight Loss and Promotes its Excretion.

Body weights of mice were measured after 4 weeks of gavage *Pediococcus acidilactici*, among which those fed on standard showed no significant difference with those fed on standard chow mixed with Cr (VI) and GR-66. Both were about 8 g heavier than the one fed on Cr (VI)-mixed standard chow with the same amount of food intake over the 4 weeks, which suggests that the decline in the weight-growth rate of mice was induced by Cr (VI) rather than the difference in the amount of food intake (Fig.2 A B). After 4-week feeding on different chow, the Cr (VI) content in the small intestine of the mice exposed to Cr (VI) and treated with GR-66 was about half of the mice solely exposed to Cr (VI). The Cr (VI) content in faces rose from 4.18 mg/g faeces to 6.81 mg/g faeces (Fig.2 C and D). Therefore, GR-66 greatly facilitated the Cr (VI) descended in the small intestine and ascended in the faeces.

2.3 *Pediococcus acidilactici* GR-66 Treatment Alleviated the Cr (VI)-induced Inflammation and Oxidative Stress.

Physiological and biochemical indexes of tissue were measured after the sacrifice of mice, which includes tumour necrosis factor-α (TNF-α), catalase (CAT), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px). Compared with the Cr (VI) group and the control group, the concentration of TNF-α in the Gr-66+Cr (VI) group decreased to 54.2 pg/mL (Fig.3 A). The GSH-Px activity of Cr (VI) group was decreased in the small intestine of mice to 2188.63 U/mg prot, while GR-66+Cr (VI)

group showed no significance compared with the control group (Fig.3 B). Moreover, the MDA concentration of the GR-66+Cr (VI) group was reduced from 3.21 nmol/mL to about 2.67 nmol/mL and the CAT activity of the GR-66+Cr (VI) group showed no significance with the control group (Fig.3 C D). In light of the above, GR-66 demonstrated the capability of efficiently attenuating inflammation and releasing oxidative stress.

2.4 *Pediococcus acidilactici* GR-66 Treatment Affected the Level of Faecal Short-Chain Fatty Acids.

Short-chain fatty acids (SCFAs), butyrate, propionate, and acetate, in faeces were measured, among which, the acetate concentration of the Gr-66+Cr (VI) group was about 9. 81 mg/g faeces, only 1 mg/g faeces lower than the control group. The concentration of propionate after GR-66 treatment showed no significance compared with the control group, while the concentration of butyrate showed no significance compared with Cr (VI) group (Fig. 4). Hence, GR-66 assisted in the maintenance of acetate and propionate concentration but have little impact on butyrate concentration.

2.5 Comp998 was Identified as a New Reductase.

After aligning the sequence of unigene with non-redundant (NR) protein in the NCBI database, GR-66 treatment upregulated Cr (VI)-reducing genes of enteric microorganisms, among which comp 998 displayed the highest relative expression level that was 6357.0470 TPM. Additionally, the NR description of comp998 was flavin reductase (Table.1). To further explore the protein encoded by comp998, comp998 was expressed by *E. coli* (BL21) and manifested a strong ability to reduce Cr (VI), of which the Cr (VI) removal rate was 77.2% and showed the highest reducing ability at 37°C and 7.3 pH (Fig.5). Considered together, the protein encoded by comp998 was identified as a reductase that shares the similar working mechanism of reducing Cr (VI) with flavin reductase.

2.6 Discussion and Conclusion

The antioxidant capability is one of the key properties of probiotics that enables them to play an essential role in intestinal enzymatic defences and repair systems (Liu et al., 2014; Wang et al., 2017). Through gene sequencing, *P. acidilactici* GR-66 possess an array of antioxidant systems, including thioredoxin, flavoprotein, flavodoxin, glutathione, and CAT (Table.1), among which thioredoxin is the dominant system to resist oxidative stress (Lu & Holmgren, 2014). CAT and glutathione are indispensable in the antioxidant defences, especially in scavenging superoxide (Ighodaro & Akinloye, 2019). Flavoprotein and flavodoxin respectively act as an antioxidant and an electron carrier in the host to improve the tolerance to oxidative stress.

Oxidative stress is a significant effect induced by the heavy metals (Tchounwou et al., 2012). Oxidative stress induces the imbalance between prooxidants and antioxidants in cells due to ROS like hydroxyl radical, superoxide and hydrogen peroxide (Ďuračková, 2010). Similar to the previous research (Karaulov et al., 2019), Cr (VI) causes severe oxidative stress in the liver, which effects can be evidently reduced by *P. acidilactici* GR-66 can boost activity of antioxidant enzymes, suppress free radicals, scavenge lipid peroxidation and reduce DNA damage in tissue (Abdellatef & Khalil, 2016; Castex et al., 2009).

Since the colonisation number of GR-66 is less than 1% relative to the gut microbiota, GR-66 limitedly contributes to the direct detoxification of Cr (VI). Thus, the alleviation of Cr (VI)-induced oxidative stress may attribute to the adjustment of gut microbiota by GR-66 (Ji et al., 2019; Wu et al., 2018). In this study, we observed that GR-66 has been involved in the increase in the number of gut microbiota and the expression of antioxidant-related genes. Among those antioxidant systems, thioredoxin and flavodoxin can convert Cr (VI) into less toxic Cr (III). More specifically, thioredoxin has participated in Cr(VI) reduction in various species (Sannasimuthu & Arockiaraj, 2019), while flavodoxin is a reductase combined with flavin mononucleotide (FMN) (Suzuki et al., 1992) that has been reported able to reduce Cr(VI) (Eswaramoorthy et al., 2012). Moreover, rubrerythrin, peroxiredoxin, superoxide dismutase (SOD) and CAT may be directly or indirectly involved in the

removal of radical scavenging (Pedone et al., 2020). H_2O_2 reducing ability of rubrerthrin has been proven (Sztukowska et al., 2002); Peroxiredoxin is a multi-functional antioxidant enzyme that is crucial in the bacterial defense against H_2O_2 (Dubbs & Mongkolsuk, 2007). SOD and CAT are recognized as important constituent that can reduce oxidative stress and attendant cellular damage (Ighodaro & Akinloye, 2018).

Apart from those annotated differential gene expressions, the functions of numerous genes are still unknown and elusive. This study, *E. coli* that expressed unknown gene comp 998 possesses greater Cr (VI)-reduction ability. Through Model fitting, we concluded that comp 998 (Table.S3) shares a similar interacting domain with flavodoxin 2HAY (PBD entry: 10.2210/pdb2hay/pdb). Thus, we proposed that comp 998 may reduce Cr (VI) by transferring electrons through FMN.

3. Materials and Methods

Bacteria and media. *The strains* GR-66, LZ-2, XS2, XN-3 and TT2 were all isolated from fermented milk from the Tibetan plateau. All probiotics were cultured aerobically with MRS broth at 37°C.

Optimization of culture condition. Five probiotics were cultured under LB medium, MRS medium and M9 medium in aerobic or anaerobic, and stationary or shaking conditions to determine the optimal growth conditions.

Determination of the Cr (VI)-reduction ability of probiotics. After incubation for 18 h, the bacterial precipitates were collected by centrifugation (10000 X g, 4°C, 2 min) and washed with PBS buffer for three times. Then, OD600 adjusted to 1.0 using fresh medium followed by adding 1 mM Cr (VI) (K2Cr2O7 solution) and cultured at 37°C for 4 h. After centrifugation, the supernatant was collected and divided into two parts. One portion was treated with excess sodium hydroxide and centrifuged again (10000 X g, RT, 2 min) to collect the supernatant. The two samples were digested with concentrated nitric acid, and the concentrations of Gr (VI) total and Cr in the supernatant were detected by atomic absorption spectrometer.

Determination of the tolerance to the hydrogen peroxide ability. The activated probiotic strains were added to the medium containing 1mM hydrogen peroxide in 1% proportion. After overnight culture at 37°C, the OD600 value was measured by spectrophotometer.

Determination of Hydroxyl radical scavenging efficiency. After incubating at 37°C water bath for 1 h, 0.1 mL 20% (m/v) TCA and 1.0% TBA were added into the tested system (Table S1). Then, the tested system was incubated at 100°C for 15 min to detect absorbance.

Determination of 2,2-diphenylpicrylhydrazyl (DPPH) scavenging efficiency. Mixed 1.9 mL 0.12 mM DPPH solution with 0.1 mL cell-free extract and cultured in the dark for 30 min. Compared the measured Optical absorbance at a wavelength of 517 nm value before and after dark culture.

Determination of the total antioxidant capacity. Total antioxidant capacity was measured using ABTS assay kit. The Optical absorbance at 520 nm was measured and the concentration of Fe (II) indicated the reducing ability of probiotics.

Determination of sequence of the genome of *P. acidilactici* GR-66. The genome of GR-66 was extracted by the Bacterial DNA kit (TIANGEN, DP302) and sequenced, following which the sequenced genome was analyzed on the I-sanger cloud service.

Culture and preparation of oral probiotics. *P. acidilactici* GR-66 was cultured overnight and then centrifuged for collection. Bacteria precipitates were resuspended with sterile defatted milk. When the CFU reached 10^9, the suspension was divided into 10 equal portions and stored at -80°C. Stored probiotics were unfrozen in the 37°C water bath before gavage administration.

Determination of Cr (IV) concentration in tissue and faeces. The Cr (VI) concentration in standard chow was raised to 300 mg/kg by adding $K_2Cr_2O_7$. After 4 weeks, tissue and faeces were harvested and digested in 20 % concentrated nitric acid overnight. The samples were then digested at 300°C until the solution was clear. The clear solution was then diluted with ultrapure water. The Cr (VI) content was measured by Inductively coupled plasma mass spectrometry (ICP-MS, Antpedia

7800). The Cr (VI) concentrations in tissue and faeces were then calculated by dividing the wet weight by the corresponding Cr (VI) content.

Determination of the physiological and biochemical indexes of tissue. Cryopreserved tissues were weighed and immersed in 0.9% (w/v) NaCl solution. It is then ground evenly with a homogenizer. Centrifugation (4℃, 3000 X g, 10 min) to collect supernatant. According to the instructions, malondialdehyde (MDA) content, catalase (CAT) activity, (GSH-px) activity, and tumor necrosis factor-α (TNF-α) content were measured using commercial assay kit.

Determination of colonization level of the GR-66. The copy numbers of *Idh*D (*P. acidilactici* specific gene) and tot (total bacteria 16S rDNA gene) fragments in fecal total genomic DNA were determined by qPCR to calculate the proportion of *P. acidilactici* GR-66 to total bacteria.

Determination of Cr (VI)-reduction ability in the intestine. 1 g faeces were collected and mixed with 10 mL sterile PBS buffer. After 2 min static settlement, 1 mL supernatant was inoculated to 100mL sterile 1 m Gr (VI) medium and anaerobically cultured at 37°C in the incubator. The OD_{600} was tested every 2 to 3 h.

Determination of antioxidant capacity in the intestine. 1 g faeces were collected and mixed with 10 mL sterile PBS buffer. After 2-min static settlement, 1 mL supernatant was collected to detect the total antioxidant capability and DPPH scavenging ability.

Determination of short-chain fatty acid concentration in the faeces. Gas chromatography was used to analyze the contents of short-chain fatty acids (SCFAs) in the faeces. 600 mg harvested faeces were collected and resuspended with 1.2 mL PBS buffer, the suspension of which was centrifuged (15000 X g, 4°C, 15 min). The supernatant was collected and filtered by 0.22 μm membranes. 800 μL supernatant was acidified filtered with 0.4 mL 50% (v/v) sulfuric acid and mixed by vortex. Organic acids contained in the samples were extracted by adding 0.4 mL diethyl ether. The SCFAs concentration was detected by a gas chromatograph (ThermoFisher Scientific, Trace 1300, Singapore) equipped with a capillary column (30 m × 0.25 mm × 0.25 μm, TG-WAXMS). Detector was set at 250°C and injector was set at 200°C. The column temperature was kept at 40°C for 5 min, the temperature was then increased at the rate of 5°C/min to 300°C. The concentration of butyrate, propionate and acetate were calculated respectively according to their standard curves.

Metatanscriptomic sequencing and analysis. Fresh feces were collected and stored at -80°C until use. In short, RNA was extracted from feces using the RNeasy Mini Kit (Qiagen, German), then RNA purity and quality were verified by Agilent 2100 (RNA integrity number should be greater than 7.0) and Nanodrop. Before library construction, rRNA was removed using ribosome RNA remove kit (Qiagen, German). The retained RNA was then sequenced using the Illumina Hiseq X Ten platform. To improve reliability, low quality reads were removed using Seqprep. The host (*Mus musculus*) genome sequence was downloaded from ensemble and removed using BWA. Trimmed data was further compared to the SILVA databases to remove all rRNA sequences. The high-quality reads were then assembled using Trinity. Open reading frames (ORFs) were predicted using TransGeneScan. All ORFs was combined to establish a non-redundant gene set, which was clustered by CD-HIT software (95% recognition rate, 90% coverage rate). The longest Unigene sequence in each cluster was used for analysis.

Differential gene expression analysis. The mass of assembled sequences was estimated by aligning the assembled sequences with filtered pair-end sequences and converting the results into default parameters in RSEM. BlastP 2.2.31+ was employed to align unigene with non-redundant protein sequences in the NCBI database. Edge R, a software package, was employed to analyze the gene differential expression, of which the selection standard was $\log_2$ fold change ($\log_2$FC) ≥ 1.

Determination of Cr (VI)-reduction ability of comp998. comp998 was synthesized and subcloned to pET28a. The recombinant plasmid was transferred into *E. coli* BL21 (DE3) and verified by DNA sequencing. To determine Cr (VI) reduction capability, engineered bacteria were inoculated in LB broth containing 100 μg/ml kanamycin and cultured at 37°C until OD600 reached 0.6 ~ 0.8. Then, 1 mM Cr (VI) and 500 μM IPTG were added and induced at 16°C. After 16 h, bacteria were collected and resuspended with Tris-HCl buffer. After ultrasound, the supernatant was collected by

centrifugation for 40 min and the protein was purified using a nickel column. Subsequently, the enzyme activity of the comp998 encoded protein was measured using the 5 mL reaction system (20 mM protein, 1 mM Cr (VI) and 3 mM NADH).

Statistical analysis. By employing GraphPad 9 (USA), data are expressed as the standard error of the mean (SEM) and one-way ANOVA test with Tukey post-test was conducted to analyse the unpair groups. The significance level adopted was 0.05.

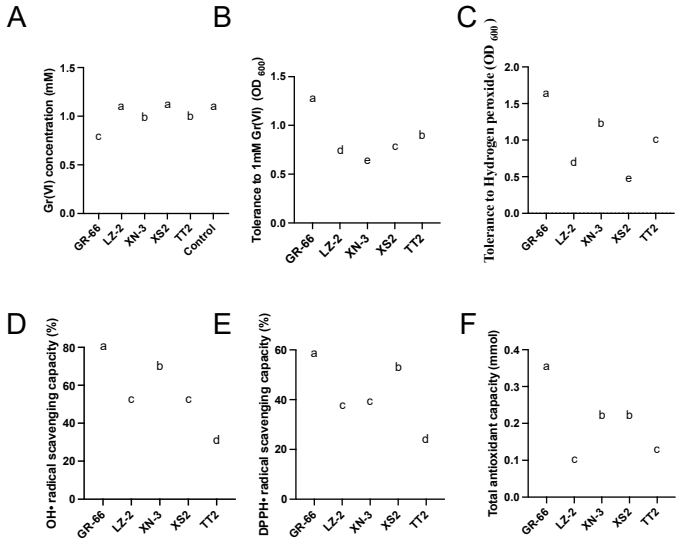


Figure 1. Gr (VI) tolerance and antioxidant abilities of probiotics GR-66, LZ-2, XS2, XN-3 and TT2. (A) Reducing capacity of probiotics to 1 mM Gr (VI). (B) Tolerance of probiotics to 1 mM Gr (VI). (C) Tolerance of probiotics to 1 mM hydrogen peroxide. (D) Hydroxyl radical scavenging capacity of probiotics. (E) DPPH· scavenging capacity of probiotics. (F) Determination of total antioxidant capacity of probiotics

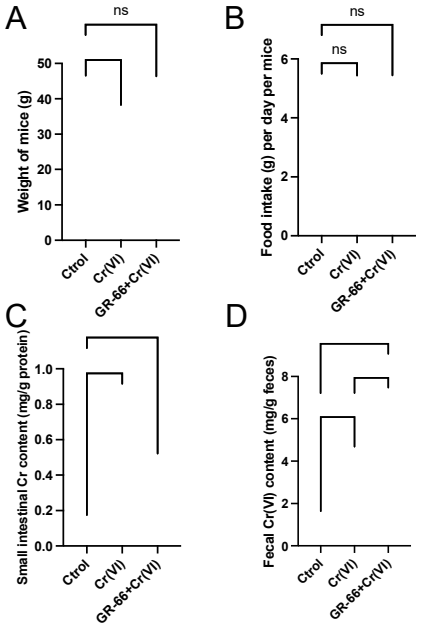


Figure 2. Mice body weight (A) and food intake (B) after 4 weeks gavage. Cr (VI) content in small intestine (C) and feces (D) of mice in each group.

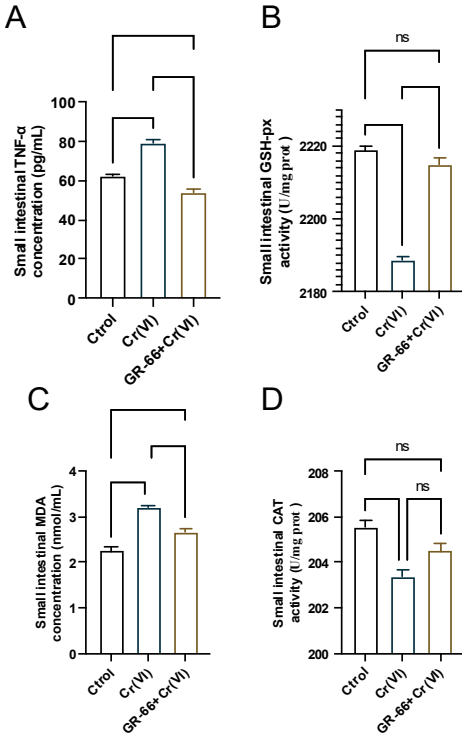


Figure 3. (A) TNF-α, (B) GSH-px, (C) MDA and (D) CAT levels in small intestine of each group mice

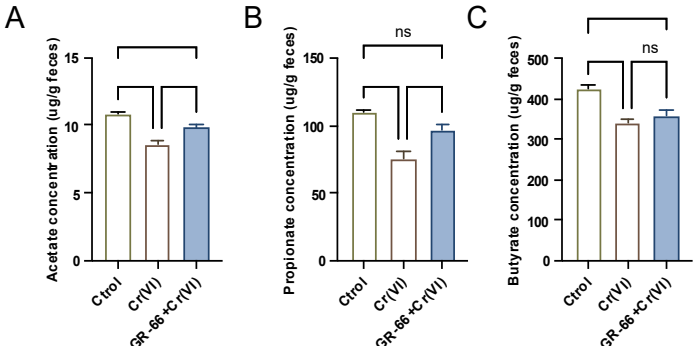


Figure 4. The concentration of fecal acetate (A), propionate (B), and butyrate (C) of different groups

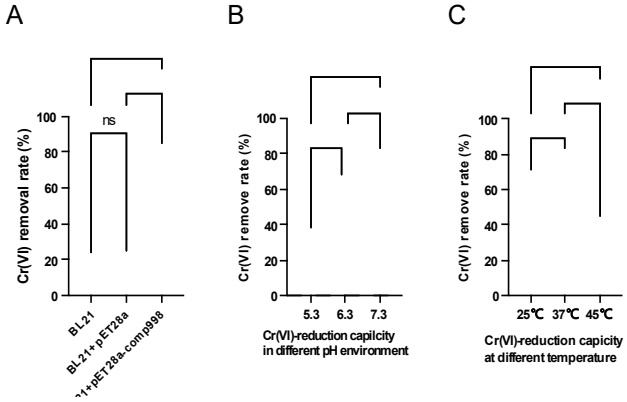


Figure 5. (A) Cr (VI) removal ability of protein coded by comp998. Gr (VI) removal ability of comp998 encoded protein at different (B) pH and (C) temperature.

Table 1. Differential gene expression between GR-66 gavage group and control groups.

Gene ID	NR description	Samples (TPM)			Fold change (log_2FC)
		GR-66+Cr (VI)	Control	Cr (VI)	
comp998	Flavin reductase	6357.0470	0	0.9497	12.7086
comp032	peroxiredoxin	2012.036	0	0	10.9744
comp086	rubredoxin	1439.4555	0	0.2006	12.8091
comp415	thiol peroxidase	1388.7344	0.1859	4.6896	8.2101
comp738	rubrerythrin	179.5021	0.8120	4.2684	5.39

Supplemental materials

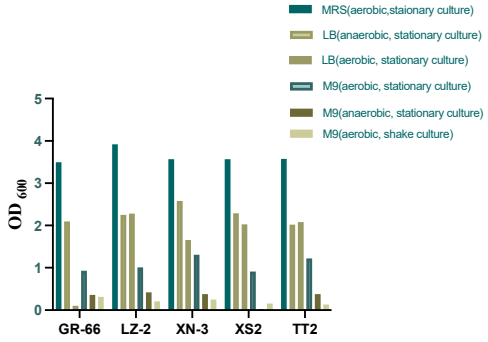


Figure 6. The growth of probiotics under different culture conditions.

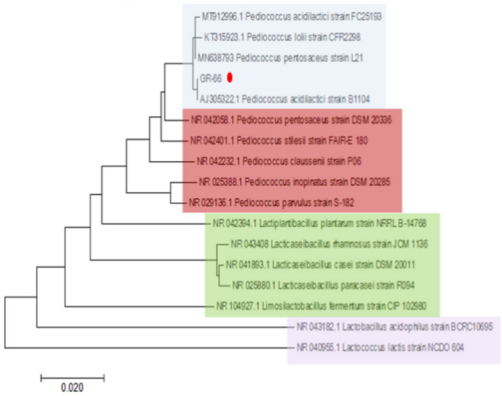


Figure 7. Phylogenetic tree of GR-66 and related bacteria.

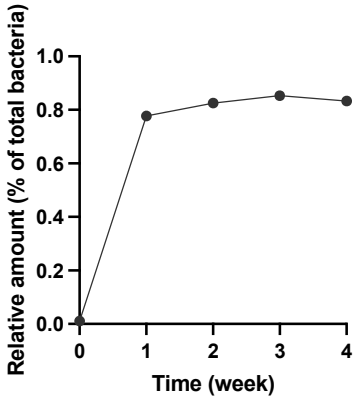


Figure 8. The colonization level of GR-66 in mice gut microbiota.

Table 2. Tested system of hydroxyl radical scavenging efficiency

cell-free extract	0.02 M PBS buffer	1mM EDTA	10 mM H_2O_2	2mM ascorbic acid	60mM 2-deoxy-D-ribose	1mM $FeCl_3$
0.1 mL	0.4 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL

Table 3. The gene that GR-66 may participate in Gr (VI) reduction.

Gene ID	NR Description	Gene Length (bp)
gene0035	catalase	693
gene0044	glutathione	623
gene0910	flavodoxin	389
gene0203	methionine sulfoxide reductase	257
gene0514	ferredoxin-NADP+ Reductase	250
gene0959	thioredoxin-disulfide reductase	352
gene0878	nitroreductase	286

Table 4. Amino acid sequence of comp998.

Gene name	Amino acid sequence
Comp998	MNQTIMDALHFRTATKQFDKEEKISDEDLALILDAAWLSPSSIGLEGWRFVVLDNKPIKEEIK PFAWGAQYQLETASHFILLIAEKHARYDSPYVQHMLLRRGIKEGDGLNSRLKLYESFQKDM DMADNDQALDWSSKQTYIALGNMMTTAALLYGIDSCPMEGFSLDTVTDILANKGILDTEQF GLSVMVAFGYRQQDPPKNKTRQAYEDVIEWVGPKE

Author Contributions

Yunyue Wang conceived the study, conducted experiments, and prepared manuscript.

Acknowledgments

This work was supported by Shenzhen Thinker Education Technology Co., LTD.

References

[1] Abdellatef, A. A., & Khalil, A. A. (2016). Ameliorated effects of Lactobacillus delbrueckii subsp. lactis DSM 20076 and Pediococcus acidilactici NNRL B-5627 on Fumonisin B1-induced Hepatotoxicity and Nephrotoxicity in rats. Asian Journal of Pharmaceutical Sciences, 11(2), 326-336. https://doi.org/ https://doi.org/ 10.1016/j.ajps.2016.02.006.

[2] Balakrishnan, R., Kumar, C. S. V. S., Reddy, K. K., Rani, M. U., Srikanth, M. K., Kavitha, K., Boobalan, G., & Reddy, A. G. (2014). Antioxidant Activity of Coated Probiotic Lactobacillus casei on Chromium(VI) Induced Oxidative Stress in Rats. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 84(2), 305-310. https://doi.org/10.1007/s40011-013-0215-5.

[3] Barnhart, J. (1997). Occurrences, uses, and properties of chromium. Regulatory toxicology and pharmacology, 26(1), S3-S7.

[4] Castex, M., Lemaire, P., Wabete, N., & Chim, L. (2009). Effect of dietary probiotic Pediococcus acidilactici on antioxidant defences and oxidative stress status of shrimp Litopenaeus stylirostris. Aquaculture, 294(3-4), 306-313. https://doi.org/10.1016/j.aquaculture.2009.06.016.

[5] Chary, N. S., Kamala, C. T., & Raj, D. S. (2008). Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicol Environ Saf, 69(3), 513-524. https://doi.org/10.1016/j.ecoenv.2007.04.013.

[6] Dubbs, J. M., & Mongkolsuk, S. (2007). Peroxiredoxins in bacterial antioxidant defense. Subcell Biochem, 44, 143-193. https://doi.org/10.1007/978-1-4020-6051-9_7.

[7] Ďuračková, Z. (2010). Some current insights into oxidative stress. Physiol Res, 59(4), 459-469. https:// doi.org/10.33549/physiolres.931844.

[8] Eswaramoorthy, S., Poulain, S., Hienerwadel, R., Bremond, N., Sylvester, M. D., Zhang, Y. B., Berthomieu, C., Van Der Lelie, D., & Matin, A. (2012). Crystal structure of ChrR--a quinone reductase with the capacity to reduce chromate. PLoS One, 7(4), e36017. https://doi.org/ 10.1371/ journal. pone.0036017.

[9] FAO/WHO, R. (2001). Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria [Internet], 1-29. https://doi.org/http://www.fao.org/tempref/docrep/fao/meeting/009/y6398e.pdf [Ref list].

[10] Feng, P., Ye, Z., Kakade, A., Virk, A. K., Li, X., & Liu, P. (2018). A Review on Gut Remediation of Selected Environmental Contaminants: Possible Roles of Probiotics and Gut Microbiota. Nutrients, 11(1). https://doi.org/10.3390/nu11010022.

[11] Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54(4), 287-293. https://doi.org/ https:// doi.org/ 10.1016/ j. ajme. 2017.09.001.

[12] Ighodaro, O. M., & Akinloye, O. A. (2019). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54(4), 287-293. https://doi.org/10.1016/j.ajme.2017.09.001.

[13] Ji, J., Kakade, A., Zhang, R., Zhao, S., Khan, A., Liu, P., & Li, X. (2019). Alcohol ethoxylate degradation of activated sludge is enhanced by bioaugmentation with Pseudomonas sp. LZ-B. Ecotoxicol Environ Saf, 169, 335-343. https://doi.org/10.1016/j.ecoenv.2018.11.045.

[14] Kamaludeen, S. P., Megharaj, M., Juhasz, A. L., Sethunathan, N., & Naidu, R. (2003). Chromium-microorganism interactions in soils: remediation implications. Reviews of Environmental Contamination and Toxicology, 93-164.

[15] Karaulov, A. V., Renieri, E. A., Smolyagin, A. I., Mikhaylova, I. V., Stadnikov, A. A., Begun, D. N., Tsarouhas, K., Buha Djordjevic, A., Hartung, T., & Tsatsakis, A. (2019). Long-term effects of chromium on morphological and immunological parameters of Wistar rats. Food Chem Toxicol, 133, 110748. https:// doi.org/10.1016/j.fct.2019.110748.

[16] Liu, J., Xin, Z., Huang, Y., & Yu, J. (2022). Climate suitability assessment on the Qinghai-Tibet Plateau. Sci Total Environ, 816, 151653. https://doi.org/10.1016/j.scitotenv.2021.151653.

[17] Liu, Y., Li, Y., Liu, K., & Shen, J. (2014). Exposing to cadmium stress cause profound toxic effect on microbiota of the mice intestinal tract. PLoS One, 9(2), e85323. https://doi.org/ 10.1371/ journal. pone.0085323.

[18] Liu, Y.-G., Xu, W.-H., Zeng, G.-M., Li, X., & Gao, H. (2006). Cr(VI) reduction by Bacillus sp. isolated from chromium landfill. Process Biochemistry, 41(9), 1981-1986. https://doi.org/ https:// doi.org/ 10.1016/ j. procbio.2006.04.020.

[19] Lu, J., & Holmgren, A. (2014). The thioredoxin antioxidant system. Free Radic Biol Med, 66, 75-87. https:// doi.org/10.1016/j.freeradbiomed.2013.07.036.

[20] Luo, H., Lu, Y., Shi, X., Mao, Y., & Dalal, N. S. (1996). Chromium (IV)-mediated Fenton-like reaction causes DNA damage: implication to genotoxicity of chromate. Annals of Clinical & Laboratory Science, 26(2), 185-191.

[21] Madhavi, V., Reddy, A. V. B., Reddy, K. G., Madhavi, G., & Prasad, T. (2013). An overview on research trends in remediation of chromium. Research Journal of Recent Sciences, 2277, 2502.

[22] Mishra, J., Singh, R., & Arora, N. K. (2017). Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Frontiers in microbiology, 8, 1706.

[23] Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A., Idris, A. M., Khandaker, M. U., Osman, H., Alhumaydhi, F. A., & Simal-Gandara, J. (2022). Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University - Science, 34(3). https://doi.org/10.1016/j.jksus.2022.101865.

[24] Oliveira, H. (2012). Chromium as an Environmental Pollutant: Insights on Induced Plant Toxicity. Journal of Botany, 2012, 1-8. https://doi.org/10.1155/2012/375843.

[25] Pedone, E., Fiorentino, G., Bartolucci, S., & Limauro, D. (2020). Enzymatic Antioxidant Signatures in Hyperthermophilic Archaea. Antioxidants (Basel), 9(8). https://doi.org/10.3390/antiox9080703.

[26] Sannasimuthu, A., & Arockiaraj, J. (2019). Intracellular free radical scavenging activity and protective role of mammalian cells by antioxidant peptide from thioredoxin disulfide reductase of Arthrospira platensis. Journal of Functional Foods, 61, 103513. https://doi.org/ https://doi.org/ 10.1016/ j.jff. 2019. 103513.

[27] Shanker, A. K., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. Environ Int, 31(5), 739-753. https://doi.org/10.1016/j.envint.2005.02.003.

[28] Shi, X., Mao, Y., Knapton, A. D., Ding, M., Rojanasakul, Y., Gannett, P. M., Dalal, N., & Liu, K. (1994). Reaction of Cr (VI) with ascorbate and hydrogen peroxide generates hydroxyl radicals and causes DNA damage: role of a Cr (IV)-mediated Fenton-like reaction. Carcinogenesis, 15(11), 2475-2478.

[29] Shi, X., Sun, X., Gannett, P. M., & Dalal, N. (1992). Deferoxamine inhibition of Cr (V)-mediated radical generation and deoxyguanine hydroxylation: ESR and HPLC evidence. Archives of biochemistry and biophysics, 293(2), 281-286.

[30] Sun, H., Brocato, J., & Costa, M. (2015). Oral Chromium Exposure and Toxicity. Curr Environ Health Rep, 2(3), 295-303. https://doi.org/10.1007/s40572-015-0054-z.

[31] Suzuki, T., Miyata, N., Horitsu, H., Kawai, K., Takamizawa, K., Tai, Y., & Okazaki, M. (1992). NAD(P)H-dependent chromium (VI) reductase of Pseudomonas ambigua G-1: a Cr(V) intermediate is formed during the reduction of Cr (VI) to Cr (III). J Bacteriol, 174(16), 5340-5345. https://doi.org/ 10. 1128/ jb.174.16.5340-5345.1992.

[32] Sztukowska, M., Bugno, M., Potempa, J., Travis, J., & Kurtz, D. M., Jr. (2002). Role of rubrerythrin in the oxidative stress response of Porphyromonas gingivalis. Mol Microbiol, 44(2), 479-488. https:// doi.org/ 10.1046/j.1365-2958.2002.02892.x.

[33] Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. Exp Suppl, 101, 133-164. https://doi.org/10.1007/978-3-7643-8340-4_6.

[34] Tsapakos, M. J., & Wetterhahn, K. E. (1983). The interaction of chromium with nucleic acids. Chemico-biological interactions, 46(2), 265-277.

[35] Unceta, N., Seby, F., Malherbe, J., & Donard, O. F. (2010). Chromium speciation in solid matrices and regulation: a review. Anal Bioanal Chem, 397(3), 1097-1111. https://doi.org/10.1007/s00216-009-3417-1.

[36] Upreti, R. K., Sinha, V., Mishra, R., & Kannan, A. (2011). In vitro development of resistance to arsenite and chromium-VI in Lactobacilli strains as perspective attenuation of gastrointestinal disorder. J Environ Biol, 32(3), 325-332.

[37] Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y., & Li, W. (2017). Antioxidant Properties of Probiotic Bacteria. Nutrients, 9(5). https://doi.org/10.3390/nu9050521.

[38] Wu, H., Shen, J., Jiang, X., Liu, X., Sun, X., Li, J., Han, W., Mu, Y., & Wang, L. (2018). Bioaugmentation potential of a newly isolated strain Sphingomonas sp. NJUST37 for the treatment of wastewater containing highly toxic and recalcitrant tricyclazole. Bioresour Technol, 264, 98-105. https:// doi.org/ 10.1016/ j. biortech.2018.05.071.

[39] Younan, S., Sakita, G. Z., Albuquerque, T. R., Keller, R., & Bremer-Neto, H. (2016). Chromium (VI) bioremediation by probiotics. J Sci Food Agric, 96(12), 3977-3982. https://doi.org/10.1002/jsfa.7725.