Selenium-enriched *Bacillus paralicheniformis* SR14 attenuates H_2O_2-induced oxidative damage in porcine jejunum epithelial cells via the MAPK pathway

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Abstract

Oxidative stress plays a detrimental role in gastrointestinal disorders. Although selenium-enriched probiotics have been shown to strengthen oxidation resistance and innate immunity, the potential mechanism remains unclear. Here, we focused on the biological function of our material, selenium-enriched *Bacillus paralicheniformis* SR14 (Se-BP), and investigated the antioxidative effects of Se-BP and its underlying molecular mechanism in porcine jejunum epithelial cells. First, we prepared Se-BP and quantified for its selenium and bacterial contents. Then, in vitro free radical scavenging activity was measured to evaluate the potential antioxidant effect of Se-BP. Third, to induce an appropriate oxidative stress model, we adopted different concentrations of H_2O_2 and determined the most suitable concentration by a methyl thiazolyl tetrazolium (MTT) assay. Regarding treatment with Se-BP and H_2O_2, we found that Se-BP increased cell viability and prevented lactate dehydrogenase release when administered prior to H_2O_2 exposure. Additionally, Se-BP markedly suppressed reactive oxygen species and malondialdehyde production in cells and effectively attenuated apoptosis. Compared with incubation with H_2O_2 alone, treatment with Se-BP significantly promoted phosphorylation of ERK and p38 MAPK signaling molecules. When administered with ERK and p38 MAPK inhibitors, Se-BP did not alleviate the decrease in cell viability. Our results suggest that Se-BP prevents H_2O_2-induced cell damage by activating the ERK/p38 MAPK signaling pathways.

Keywords Selenium-enriched *Bacillus paralicheniformis* SR14 · H_2O_2 · Oxidative stress · MAPK · Porcine jejunum epithelial cells

Introduction

Oxidative stress basically defines a state of imbalance in which the pro-oxidant–antioxidant balance in cells is disturbed (Bhatti et al. 2017). Excessive or sustained production of reactive oxygen species (ROS) and/or a deficiency of antioxidants results in endogenous oxidative stress, which causes DNA hydroxylation, protein denaturation, lipid peroxidation, and mitochondrial damage and apoptosis, ultimately compromising cell viability (Balaban et al. 2017; Cheng et al. 2017; Nathan and Cunningham-Bussel 2013; Song et al. 2017), and also promotes various diseases, such as inflammatory bowel disease (Bhattacharyya et al. 2014) and gastrointestinal cancers (Aw 1999). Many studies have reported that oxidative stress is usually accompanied by the production of ROS, including hydroxyl radicals and the superoxide radical (Ray et al. 2012). Thus, effective and timely elimination of ROS is beneficial for alleviating oxidative stress.

Studies have reported that ROS can directly regulate protein kinases and phosphatases in mitogen-activated protein kinase (MAPK) cascades (Ray et al. 2012). MAPKs regulate

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diverse cellular programs, including embryogenesis, proliferation, and cell apoptosis. The most well-known MAPKs are the extracellular signal-regulated kinases 1 and 2 (ERK1/2), the c-Jun N-terminal kinases (JNK1, JNK2, and JNK3), and the p38 (α, β, γ, and δ) family. These kinases are evolutionarily conserved in eukaryotes and play pivotal roles in cellular responses related to various pathways (Raman et al. 2007).

In the search for approaches to effectively protect the body against oxidative damage, an important trace element, selenium, attracted researchers' attention. Selenium, initially described as a toxin, was subsequently shown to be an essential antioxidant (Hatfield et al. 2014) that plays a key role in redox regulation, antioxidant defense, and immune function through glutathione peroxidase (GPx) enzymes and thioredoxin reductase, which catalyze the removal of excess, potentially damaging radicals produced under stress conditions (Danesi et al. 2006; Maggini et al. 2007). In recent years, several studies have reported that selenium and selenium-enriched materials can relieve various types of oxidative damage caused by inducers such as H_2O_2 and some toxins (Balaban et al. 2017; Guan et al. 2014; Ravn-Haren et al. 2008; Xu et al. 2007; Zhang et al. 2011).

Moreover, probiotics, nonpathogenic microorganisms that can resist digestion in the host small intestine and reach the colon alive, have been reported to exert a myriad of beneficial effects, including balancing the intestinal microbiota, accelerating growth, promoting the immune response, and inhibiting invasion by pathogenic bacteria (Musa et al. 2009; Yang et al. 2009). Thus, probiotics have attracted attention for their beneficial effects on animal health.

It might be worthwhile to explore whether the combination of both treatments would have a synergistic effect on body health. Earlier studies reported that some special probiotics not only performed advantageous roles in promoting body health but also resisted hostile environments with excess toxins, such as selenite and selenate, disturbing the original balance. These probiotics could transform inorganic selenium to organic or red elemental selenium, aiming to lowering its toxicity (Mccready et al. 1966). In contrast, selenium enhances the bioactivity of probiotics, and this complex possesses the beneficial effects of both agents. To date, many kinds of selenium-enriched probiotics have been identified, but few studies, mainly in pigs and rats, have reported that selenium-enriched probiotics could enhance the body's antioxidant capability and immune function, protect against various oxidative stresses, and inhibit pathogenic bacteria (Chen et al. 2005; Gan et al. 2013; Nido et al. 2016; Pescuma et al. 2017; Yazdi et al. 2013). Almost no studies of selenium-enriched probiotics have focused on oxidative damage to cells. Thus, it is necessary to consider the effects of selenium-enriched probiotics on the cell antioxidative status and immune function and to investigate its potential mechanism.

To the end, we have recently developed a bacterium, *Bacillus paralicheniformis* SR14, which can resist high concentrations of selenium and effectively transform sodium selenite to elemental selenium and organic selenium as we explained previously (Cheng et al. 2017). These special bacteria have attracted our interest for use in the mixture of a probiotic and low-toxicity selenium.

Therefore, the objective of this study was to investigate the potential effects of selenium-enriched *B. paralicheniformis* SR14 (Se-BP) and *B. paralicheniformis* SR14 (BP) on H_2O_2-induced oxidative stress in IPEC-J2 cells in vitro and elucidate the underlying molecular mechanisms.

Materials and Methods

Chemicals and Reagents

Phosphate buffer powder, 30% hydrogen peroxide (H_2O_2), glucose, NaCl, K_2HPO_4, and $MgSO_4$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Yeast extract and tryptone were purchased from OXOID (Hampshire, UK). Tertiary butylhydroquinone (TBHQ), DPPH, dimethyl sulfoxide (DMSO), and sodium selenite were purchased from Sigma-Aldrich (St. Louis, USA). The MTT cytotoxicity detection kit, whole-cell lysis assay kit, BCA assay kit, and ECL luminescence reagent were purchased from KeyGEN BioTECH (Nanjing, China). The FITC Annexin V Apoptosis Detection Kit was purchased from BD Biosciences (San Diego, USA). The GSH-Px ELISA Kit and TrxR ELISA Kit were purchased from Sangon Biotech (Shanghai, China). The Total Antioxidant Capacity Assay Kit with ABTS Method, Reactive Oxygen Species Assay Kit, Lipid Peroxidation MDA Assay Kit, Catalase Assay Kit, Total Superoxide Dismutase Assay Kit with NBT, and Lactate Dehydrogenase (LDH) Cytotoxicity Assay Kit were purchased from Beyotime Biotechnology (Shanghai, China). The Inhibition and Production of Superoxide Anion Assay Kit and Hydroxyl Free Radical Assay Kit were purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China). Primary antibodies against p38 MAPK (9212), phospho-p38 (4511), SAPK/JNK (9252), and phospho-JNK (4668) were purchased from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies against ERK1/2 (ab17942), phospho-ERK1/2 (ab76299), and β-actin (ab8226) and secondary anti-rabbit IgG (ab6721) antibodies were purchased from Abcam (Cambridge, MA). The secondary anti-mouse IgG (E030110-01) antibody was purchased from EarthOx (San Francisco, USA). Inhibitors of p38 MAPK (SB203580), ERK1/2 (GSK1120212), and JNK (SP600125) were purchased from Selleck.cn (USA). Dulbecco's Modified Eagle Medium and Ham's F12 Nutrient Mixture (DMEM/F12) was purchased from

YOCON (Beijing, China). Fetal bovine serum (FBS) was purchased from Gemini (West Sacramento, CA).

Bacteria strains and cultivation

Bacillus paralicheniformis SR14, an exopolysaccharide-producing bacterial strain conserved by the China General Microbiological Culture Collection Center (CGMCC No. 13908), was identified and maintained in our laboratory. In this study, the cultivation process was developed according to our preliminary research (Cheng et al. 2017) with minor modifications. Briefly, to obtain two kinds of product with or without selenium, 200 μL of phosphate buffer solution (PBS) or 1 mol/L sodium selenite were added to the cultures. Both incubation time were 48 h.

Preparation and characterization of BP and Se-BP

After cultivation, 10 mL of suspension was centrifuged at room temperature and $3000 \times g$ for 10 min; the pellet was washed twice with PBS and then resuspended in 5 mL of PBS. The selenium concentration of the prepared products (Se-BP and BP) was measured according to the method described previously (Hall and Gupta 1969). The bacterial concentration was measured by the flat colony counting method.

Free radical scavenging activity

The superoxide anion radicals of Se-BP and BP were assessed according to the manufacturer's instructions (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China). Briefly, 50 μL of sample solutions at gradient concentrations (0.25, 0.5, 1, 2, and 4 mM) were mixed fully with 1.0 mL of reagent 1, 0.1 mL of reagent 2, 0.1 mL of reagent 3, and 0.1 mL of reagent 4 and were then heated in a 37 °C water bath for 40 min, followed by the addition of 2.0 mL of developer for 10 min. Absorbance was measured at a wavelength of 550 nm. The control tube contained 50 μL of double-distilled water, and the standard tube contained 50 μL of vitamin C.

The hydroxyl radical scavenging activity of Se-BP and BP was tested according to the manufacturer's instructions (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China). Briefly, 0.2 mL of sample solutions at different concentrations (0.25, 0.5, 1, 2, and 4 mM) were mixed with 0.2 mL of substrate solution and 0.4 mL of reagent 3. After the reaction proceeded for 1 min at 37 °C, 2.0 mL of developer was added immediately, and the tube was mixed and placed at room temperature for 20 min. Absorbance was measured at a wavelength of 550 nm. The control tube contained 0.4 mL of double-distilled water, and the standard tube contained 0.2 mL of H_2O_2 (0.03%).

Total antioxidant capacity was measured as recommended by the protocol (Beyotime Biotechnology, Shanghai, China).

Briefly, 200 μL of ABTS working solution was reacted with 10 μL of sample solution at different concentrations (0.25, 0.5, 1, 2, and 4 mM) for 2 to 6 min. Absorbance was measured at a wavelength of 734 nm. The standard curve was generated from different concentrations (0.15, 0.3, 0.6, 0.9, 1.2, 1.5 mM) of Trolox (standard reagent). The control tube contained 10 μL of double-distilled water.

DPPH radical scavenging activity was analyzed according to the method of Cheng et al. (2017). Briefly, 100 μL of sample solution at gradient concentrations (0.25, 0.5, 1, 2, and 4 mM) was mixed with 100 μL of freshly prepared 0.5 mM DPPH-alcohol solution in a 96-well culture plate, which was incubated in the dark at room temperature for 30 min. Absorbance was measured at a wavelength of 517 nm. The control tube contained 100 μL of double-distilled water.

Cell line and cell culture

The porcine jejunum epithelial cells (IPEC-J2), a gift from Dr. Yin Yulong (Institute of Subtropical Agriculture, The Chinese Academy of Sciences), were cultured in DMEM/F12 supplemented with 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. For subsequent experiments, IPEC-J2 cells were seeded into 6- or 96-well plates; the volume of complete medium was 2 mL for 6-well plates and 200 μL for 96-well plates. Cells were allowed to adhere for 18–24 h before treatment.

Cell treatments

To determine the antioxidant effects of Se-BP and BP, IPEC-J2 cells were divided into several groups: control, H_2O_2 treatment, Se-BP preprotection + H_2O_2 treatment, and BP preprotection + H_2O_2 treatment. Briefly, IPEC-J2 cells in the control group were cultured in DMEM-F12 (without FBS and antibiotics) for 4 h. Then, cells were washed with PBS and cultured in DMEM-F12 (without FBS) for another 10 h. IPEC-J2 cells in the H_2O_2 group were cultured in DMEM-F12 (without FBS and antibiotics) for 4 h. Subsequently, DMEM-F12 (without FBS) containing H_2O_2 (at the optimal concentration as determined by an MTT assay) was added for 10 h to induce oxidative stress. For the Se-BP/BP preprotection + H_2O_2 group, after cells were cocultured with Se-BP or BP for 4 h, PBS was used to wash away the probiotics, and cells were then cultured with H_2O_2 under the same conditions as the H_2O_2 group.

Measurement of cell viability

An MTT colorimetric assay was used to monitor cell viability. Briefly, IPEC-J2 cells were seeded into 96-well plates (2×10^4 cells per well). After treatment, cells were washed twice with PBS and incubated with 5 mg/mL MTT working solution for

4 h at 37 °C. Then, the supernatant was removed, and the culture was resuspended in 150 μL of DMSO to dissolve MTT formazan crystals, followed by mixing on a shaker for 15 min. The absorbance was measured at 570 nm using a SpectraMAX M5 (Molecular Devices, USA). The effect of Se-BP, BP, and H_2O_2 on cell viability was assessed as the percentage of viable cells in each treatment group relative to untreated control cells, which were arbitrarily assigned a viability of 100%. For the inhibition experiments, cells were pretreated with or without inhibitors of p38 MAPK (SB203580, 25 μM), ERK1/2 (GSK1120212, 10 μM), and JNK (SP600125, 20 μM) and were then treated with or without the indicated concentrations of either Se-BP or BP for the indicated duration. Next, cells were treated with H_2O_2 before cell viability was measured.

Measurement of LDH release

The release of LDH into the culture medium through damaged membranes was measured spectrophotometrically using a Lactate Dehydrogenase (LDH) Cytotoxicity Assay Kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's protocol. IPEC-J2 cells were seeded into 96-well plates (2×10^4 cells per well). After treatment, absorbance was measured at a wavelength of 490 nm.

Measurement of intracellular ROS

Intracellular accumulation of ROS was measured using an ROS Detection Kit (Beyotime Biotechnology Shanghai, China) according to the manufacturer's instructions. Briefly, IPEC-J2 cells were cultured in a 6-well plate (1×10^5 cells per well), and the culture medium was renewed when the cells reached 80% confluence. After treatment, the cell culture medium was removed, and the cells were washed once with PBS and incubated with 10 μM 2,7-dichlorofluorescin diacetate (DCFH-DA) probe (diluted in FBS-free medium) at 37 °C for 20 min. Then, the DCFH-DA solution was removed, and the cells were washed three times with FBS-free medium. Finally, images were acquired by fluorescence microscopy (Nikon, Japan) at an excitation wavelength of 488 nm and an emission wavelength of 525 nm, and the fluorescence intensity represented the intracellular ROS level. For evaluation of ROS generation by flow cytometry (BD Biosciences, San Diego, USA), cells were collected in tubes after the appropriate treatment and stained with DCFH-DA as described above. For each sample, 15,000–20,000 events were recorded. Cells were gated on the forward scatter (FSC) and side scatter (SSC) signals before fluorescence was assessed. Cells with a fluorescence signal above the background, indicating positive ROS generation, were detected in the FL1 channel (excitation at 488 nm and emission at 525 nm).

Assessment of lipid peroxidation

The content of malondialdehyde (MDA) was measured by a Lipid Peroxidation MDA Assay Kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's protocol. IPEC-J2 cells were cultured in a 6-well plate (1×10^5 cells per well). After treatment, cells were washed twice with cold PBS and harvested in lysis buffer containing protease inhibitors and phosphatase inhibitors (KeyGEN BioTECH, Nanjing, China). After centrifugation at $12000 \times g$ and 4 °C for 10 min, the supernatant of the suspension was collected. Protein concentrations were determined by a BCA Protein Assay Kit (KeyGEN BioTECH, Nanjing, China). Samples were measured according to the manufacturer's instructions.

Analysis of cell apoptosis

Cell apoptosis was analyzed by a FITC Annexin V Apoptosis Detection Kit (BD Biosciences, San Diego, USA) according to manufacturer's instructions. IPEC-J2 cells (1×10^5 cells per well in a 6-well plate) were treated as above. For fluorescence observation, cells were washed once with PBS and stained with Annexin V-FITC and PI in binding buffer for 15 min. The fluorescence intensity was detected by fluorescence microscopy (Nikon, Japan) within 1 h. For flow cytometric analysis, cells were trypsinized without EDTA, washed with PBS, resuspended in binding buffer, and stained with Annexin V-FITC and PI for 15 min before being analyzed with a flow cytometer (BD Biosciences, San Diego, USA) within 1 h. All procedures were performed following the manufacturer's protocol. Flow cytometry data were analyzed with Flow-Jo. The quantities of early apoptotic and late apoptotic cells are shown in the Q3 and Q2 quadrants, respectively.

Western blot analysis

IPEC-2 cells (1×10^5 cells per well in a 6-well plate) were collected, and total cellular protein was extracted using a Whole Cell Lysis Assay Kit (KeyGEN BioTECH, Nanjing, China). According to the manufacturer's protocol, cells were centrifuged at $12,000 \times g$ at 4 °C for 10 min, and supernatants were collected for the assay. Protein concentrations were determined using a standard BCA protein assay kit. Thirty micrograms of protein was loaded per sample/lane, separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, USA). Membranes were blocked with 5% nonfat dry milk solution for 1 h at room temperature. Blocked membranes were incubated with appropriate primary antibodies at 4 °C overnight and were then incubated with secondary antibodies for 1 h at room temperature. Chemiluminescence detection was performed using an ECL luminescence reagent according

to the manufacturer's instructions. Specific bands were detected, analyzed, and quantified by ImageJ software (NIH, Bethesda, MD, USA).

Statistical analyses

All data are expressed as the means ± standard deviation (SD). Quantitative analysis of fluorescence intensity was performed using ImageJ. The flow cytometry data were analyzed by Flow Jo. One-way analysis of variance (ANOVA) followed by a least significant difference multiple comparison test was used, and $P < 0.05$ was considered statistically significant. All statistical tests were carried out using SPSS 21 software.

Results

Preparation and characterization of Se-BP

As we demonstrated previously, Se-BP was prepared by supplementation with 2 mM selenium and quantified for subsequent experiments. The results showed that the dry matter contents of Se-BP and BP were 38.20 and 37.18 g/L, respectively. The bacterial counts of Se-BP and BP were 2×10^7 and 2×10^8 cfu/mL, respectively; total selenium content of Se-BP was 4132.2 mg/kg, while the elemental selenium content was only 1489.1 mg/kg, indicating that Se-BP is a complex of different forms of selenium-enriched probiotics. The detailed components are unclear. Thus, we did not establish a selenium control group. In the following experiments, the concentrations of bacteria and selenium are noted.

Free radical scavenging activity of Se-BP in a cell-free system

To evaluate the ability of Se-BP to resist oxidative stress, an in vitro scavenging free radical activity assay was conducted. First, we measured the activity of superoxide radical scavenging. The results showed that both Se-BP and BP scavenged superoxide radicals in a dose-dependent manner (Fig. 1a), while Se-BP exhibited a higher scavenging ability than BP. The difference may be due to selenium addition and some exopolysaccharide production, which enhanced the scavenging capacity of Se-BP.

Second, Fig. 1b shows that Se-BP and BP almost completely removed hydroxyl free radicals. These results demonstrated that both Se-BP and BP could prevent ROS-induced cell damage.

Then, we also measured the scavenging rate of DPPH, with vitamin C as the positive control. Se-BP showed an even higher scavenging ability than vitamin C, and the capability of BP was much lower than that of Se-BP, at approximately

30% (Fig. 1c). In summary, Se-BP exhibited a higher antioxidant capacity than BP.

Finally, we evaluated another radical ABTS. The results showed that Se-BP exhibited a higher efficiency than BP in scavenging ABTS radicals in a dose-dependent manner; however, BP had almost no ability to eliminate ABTS radicals (Fig. 1d). Hence, Se-BP exhibited a higher total antioxidant capacity than BP.

In summary, both Se-BP and BP showed a certain antioxidant capacity, while Se-BP exhibited a greater potential than BP. Together, these results provided a foundation for future antioxidant research.

Preventive effect of Se-BP on H₂O₂-induced oxidative stress in IPEC-J2

To investigate whether Se-BP could prevent H_2O_2-induced oxidative stress, IPEC-J2 cells were subjected to concentrations of H_2O_2 ranging from 100 to 800 μM for 10 h. The results of cell exposure to H_2O_2 are shown in Fig. S1 (a). The viability of cells treated with 400 μM H_2O_2 for 10 h was approximately 60–80% that of the control cells. Therefore, treatment with 400 μM H_2O_2 for 10 h was selected for subsequent experiments. Regarding the concentrations of Se-BP and BP, cells were incubated with Se-BP (1-20 μM) or BP (5×10^3–1×10^5 cfu/mL) for 4 h to clarify the effect of the appropriate concentration. The results showed that Se-BP (1-20 μM) and BP (5×10^3–1×10^5 cfu/mL) had no significant effect on cell viability (Fig. S1 (b)). Thus, subsequent experiments were performed using Se-BP concentrations of ≤ 20 μM and BP concentrations of ≤ 1×10^5 cfu/mL for 4 h.

Pretreatment of IPEC-J2 cells with Se-BP (10 and 20 μM) or BP (5×10^4 cfu/mL) significantly improved cell viability in the presence of H_2O_2 (400 μM), and a low dose of Se-BP did not attenuate H_2O_2-induced cell damage (Fig. 2a), which provided us with a reference for selecting the next concentrations of Se-BP and BP.

To further explore the beneficial effect of Se-BP, the release of intracellular LDH, an indicator of cell injury, was measured in H_2O_2-exposed IPEC-J2 cells. Compared with untreated control cells, H_2O_2 group cells exhibited significantly enhanced LDH release, indicating that these cells were severely damaged by H_2O_2, while pretreatment with low doses of Se-BP (1–5 μM) or BP (5×10^3–2.5×10^4 cfu/mL) did not attenuate LDH release. However, preincubation with Se-BP (10 and 20 μM) significantly reduced the release of LDH compared with that in the H_2O_2 group and was consistent with LDH release from the untreated control cells; however, BP (5×10^4 and 1×10^5 cfu/mL) substantially decreased the level of intracellular LDH compared with that in the H_2O_2 group, but this effect did not completely resolve the cell damage relative to untreated control cells (Fig. 2b).
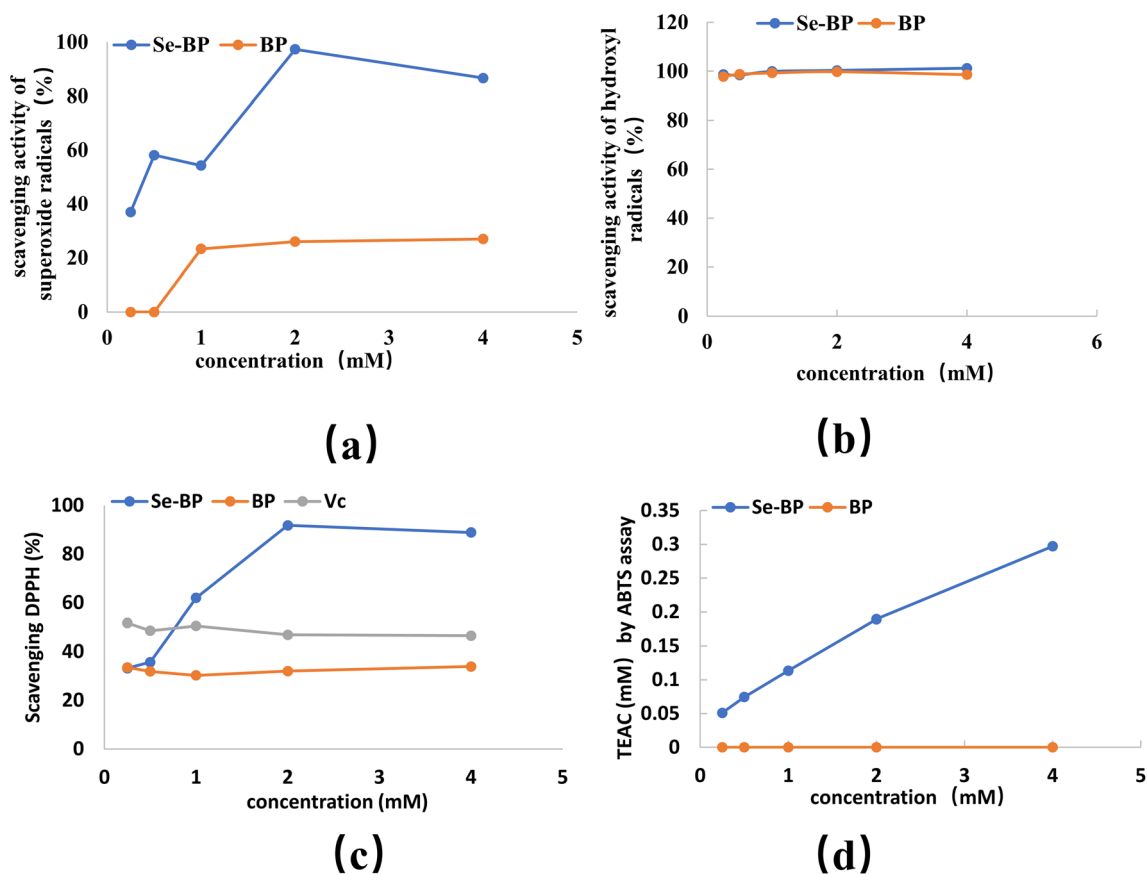
Fig. 1 Antioxidant properties of Se-BP and BP. **a** Scavenging activity against superoxide radicals, **b** hydroxyl radicals, **c** DPPH radicals (vitamin C [Vc] was used as the reference compound), and **d** ABTS radicals.

Collectively, the results of the cell viability and LDH release experiments indicate that treatment with Se-BP (10 and 20 μM) or BP (5×10^4 and 1×10^5 cfu/mL) for 4 h was beneficial for maintaining cell integrity; these concentrations were thus selected for subsequent research.

Se-BP inhibits oxidative damage-induced increases in ROS and MDA production in IPEC-J2

To validate the beneficial effect of Se-BP on ameliorating oxidative damage induced by H_2O_2, intracellular ROS production in IPEC-J2 cells was monitored by fluorescence microscopy and flow cytometry using DCFH-DA as the fluorescent probe. As shown in Fig. 3a, IPEC-J2 cells exposed to H_2O_2 (400 μM) for 10 h showed a significant increase in fluorescence, which is proportional to the amount of ROS generation. The increase in ROS generation was markedly inhibited by Se-BP (10 and 20 μM) pretreatment. Cells treated with BP (5×10^4 and 1×10^5 cfu/mL) showed a decreasing trend but no appreciable impact on ROS production compared with that seen in the H_2O_2 group. The flow cytometry results (Fig. 3b) further confirmed that Se-BP (10 and 20 μM) significantly ameliorated H_2O_2-induced ROS production, whereas BP (5×10^4 and 1×10^5 cfu/mL) barely attenuated the

intracellular level of ROS, consistent with the findings in our previous study (Cheng et al. 2017; Song et al. 2017).

To investigate the effect of Se-BP on lipid peroxidation in cultured IPEC-J2 cells, MDA was measured according to an established protocol. As shown in Fig. 3c, the MDA level was markedly increased in response to H_2O_2 compared to that in untreated control cells, whereas pretreatment with Se-BP (10 and 20 μM) significantly inhibited MDA production, and BP (5×10^4 cfu/mL) did not attenuate the MDA levels. In conclusion, Se-BP but not BP effectively protected against H_2O_2-induced oxidative damage, which further indicates that Se-BP has a higher antioxidant capacity than BP in vitro. These results have been shown in vivo (Chen et al. 2005).

The inhibition of H_2O_2-induced ROS generation and MDA by pretreatment with Se-BP may occur via a direct antioxidant mechanism through free radical scavenging activity, similar to the results shown in Fig. 1; however, the definite mechanism needs further investigation.

Se-BP prevents H_2O_2-induced apoptosis of IPEC-J2 cells

We detected apoptosis by fluorescence microscopy and flow cytometry based on Annexin V-FITC/PI double staining.

(a)

(b)
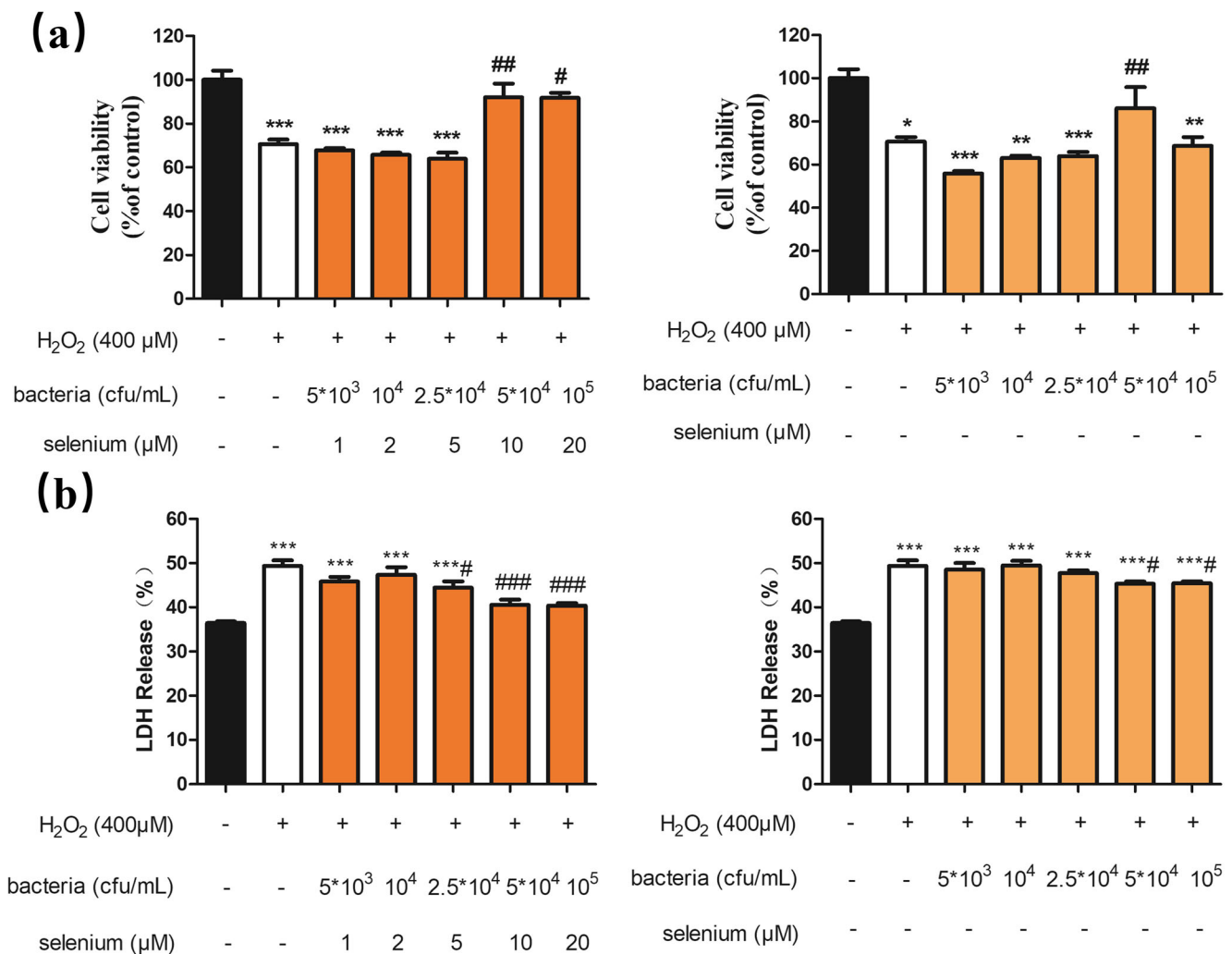
Fig. 2 Se-BP inhibits hydrogen peroxide (H_2O_2)-induced cytotoxicity in IPEC-J2 cells. Cells in the control group (the black bars) were cultured in DMEM-F12 (without FBS and antibiotics) for 4 h, and then were washed with PBS before being cultured in DMEM-F12 (without FBS) for another 10 h. IPEC-J2 cells in the H_2O_2 group (the white bars) were cultured in DMEM-F12 (without FBS and antibiotics) for 4 h and DMEM-F12 (without FBS) containing 400 μM H_2O_2 was then added for 10 h. IPEC-J2 cells in the Se-BP/BP preprotection + H_2O_2 group were preculured with Se-BP (1–20 μM) or BP (5×10^3–10^5 cfu/mL) for 4 h and incubated with 400 μM H_2O_2 for 10 h. **a** Cell viability was determined by MTT assays. **b** LDH release was measured by an LDH Cytotoxicity Assay Kit. The values are the means ± SDs (*$P < 0.05$, compared with untreated control cells; #$P < 0.05$, compared with the H_2O_2 group). Significant differences between the groups were analyzed with one-way ANOVA

Qualitative analysis showed that H_2O_2 (400 μM) significantly increased the number of apoptotic cells compared to that in the untreated control group, and the increase in apoptosis was markedly inhibited by Se-BP (10 μM and 20 μM), but only slightly alleviated by BP at 5×10^4 cfu/mL and 1×10^5 cfu/mL (Fig. 4a). Further results are shown in Fig. 4b, c. The upper right quadrant (Q2) and lower right quadrant (Q3) represent the populations of late apoptotic cells (PI staining) and early apoptotic cells (Annexin V-FITC staining), respectively. The H_2O_2 group contained a high percentage of apoptotic cells, whereas the Se-BP group contained a significantly lower percentage of apoptotic cells, indicating the protective effect of Se-BP against the apoptosis enhancement. Quantitative analysis results showed that Se-BP (10 μM and 20 μM)

appreciably decreased H_2O_2-induced cell apoptosis to levels similar to those in the untreated control group; BP (5×10^4 cfu/mL, 1×10^5 cfu/mL) markedly enhanced cell apoptosis compared with that seen in the control group, and BP (1×10^5 cfu/mL) was beneficial for decreasing H_2O_2-induced cell apoptosis. Taken together, these results indicate that Se-BP (10 μM and 20 μM) and BP (1×10^5 cfu/mL) effectively ameliorate H_2O_2-induced cell apoptosis.

Activation of MAPKs by Se-BP

To explore the possible mechanism underlying the antioxidant effects of Se-BP, we focused on the role of the MAPK pathway, which has also been reported to regulate NP cell
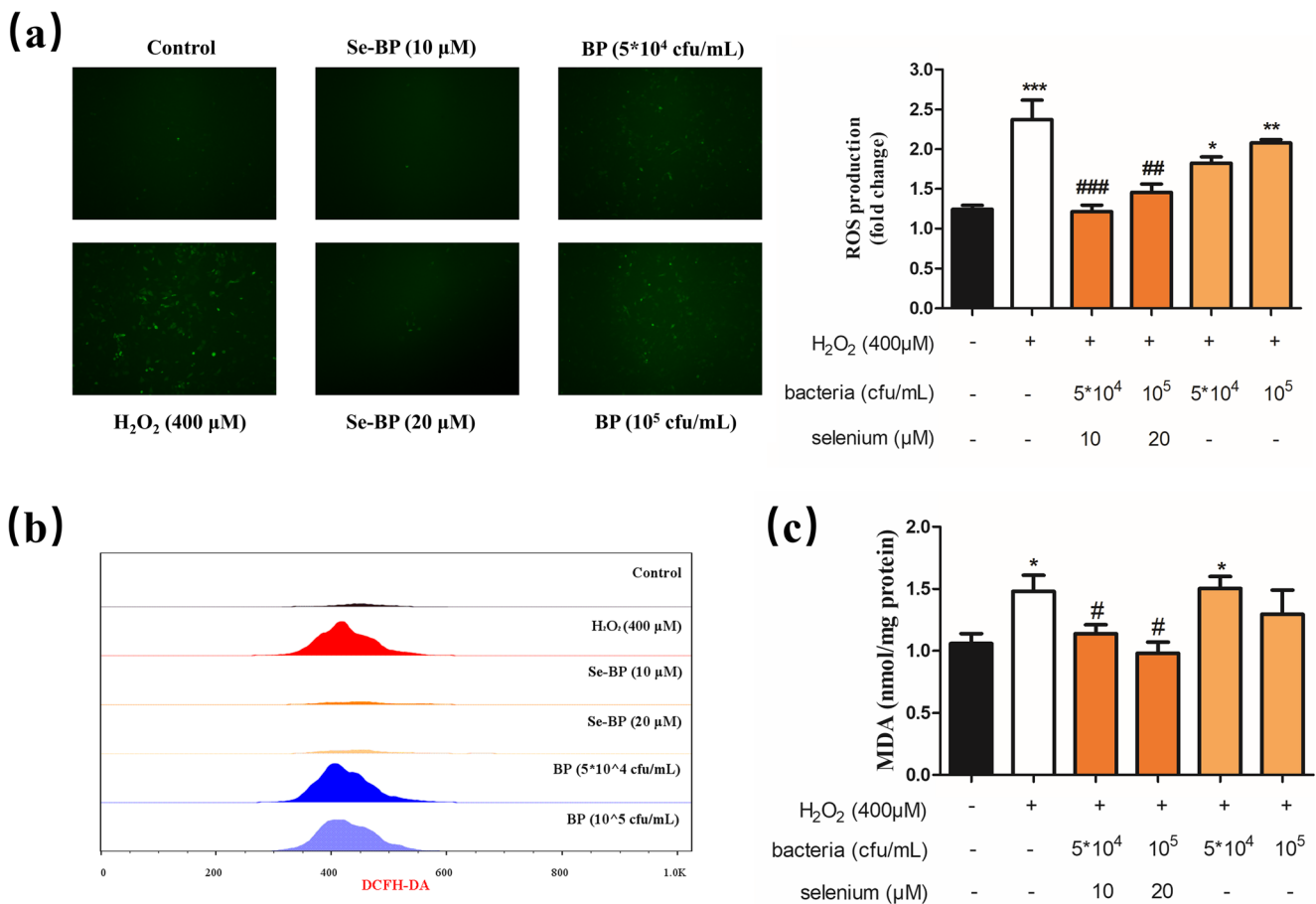
Fig. 3 Effects of Se-BP on ROS and MDA production in damaged IPCE-J2 cells. Cells in the control group (the black bars) were cultured in DMEM-F12 (without FBS and antibiotics) for 4 h, and were then washed with PBS before being cultured in DMEM-F12 (without FBS) for another 10 h. IPEC-J2 cells in the H_2O_2 group (the white bars) were cultured in DMEM-F12 (without FBS and antibiotics) for 4 h and then DMEM-F12 medium (without FBS) containing 400 μM H_2O_2 was then added for 10 h. IPEC-J2 cells in the Se-BP/BP preprotection + H_2O_2 group were precultured with Se-BP (10 μM and 20 μM) or BP (5×10^4 cfu/mL, 10^5 cfu/mL) for 4 h and incubated with 400 μM H_2O_2 for 10 h. **a**, **b** Intracellular ROS levels were measured by fluorescence microscopy (**a**) and flow cytometry (**b**). **c** Intracellular MDA levels were measured. The values are the means ± SDs (*$P < 0.05$, compared with untreated control cells; #$P < 0.05$, compared with the H_2O_2 group). Significant differences between the groups were analyzed with one-way ANOVA

senescence downstream of ROS (Feng et al. 2017). In this study, we discovered that treatment with Se-BP (20 μM) for 4 h significantly increased the phosphorylation levels of ERK and p38 MAP kinases but had no effect on the total protein levels of JNK, ERK, and p38 (Fig. 5a), indicating that Se-BP activated the ERK/p38 MAPK signaling pathway. However, BP had no effect on the levels of total and phosphorylated MAPKs. These results demonstrate that Se-BP ameliorates oxidative stress more effectively than BP by activating the MAPK pathway. To further confirm this conclusion, we adopted inhibitors of p38 (SB203580), ERK (GSK1120212), and JNK (SP600125). Figure S2 (a and b) shows that these inhibitors did not impact cell viability at the tested concentrations and effectively prevented the expression of relevant proteins. In addition, Fig. 5b, c clearly demonstrates that Se-BP did not promote the phosphorylation of MAPKs and attenuated the decrease in cell viability when combined with the inhibitors. These results further verified

that in the presence of MAPK inhibitors, Se-BP cannot effectively activate the ERK/p38 MAPK pathway to protect against oxidative damage.

Discussion

Previous studies have indicated that oxidative stress is involved in intestinal injury (Bhattacharyya et al. 2014) in vivo and in vitro, which severely damages the development of animal husbandry. H_2O_2 is one of the most well-known factors that causes severe oxidative stress (Liu et al. 2012; Zou et al. 2016), further damaging intestinal homeostasis. It is well documented that exposure to H_2O_2 triggers the rapid generation of ROS in IPEC-J2 cells. Accumulation of ROS and impairment of the antioxidant defense system by H_2O_2 causes oxidative damage in cells (Je and Lee 2015). Hence, we established an H_2O_2-induced oxidative stress model in
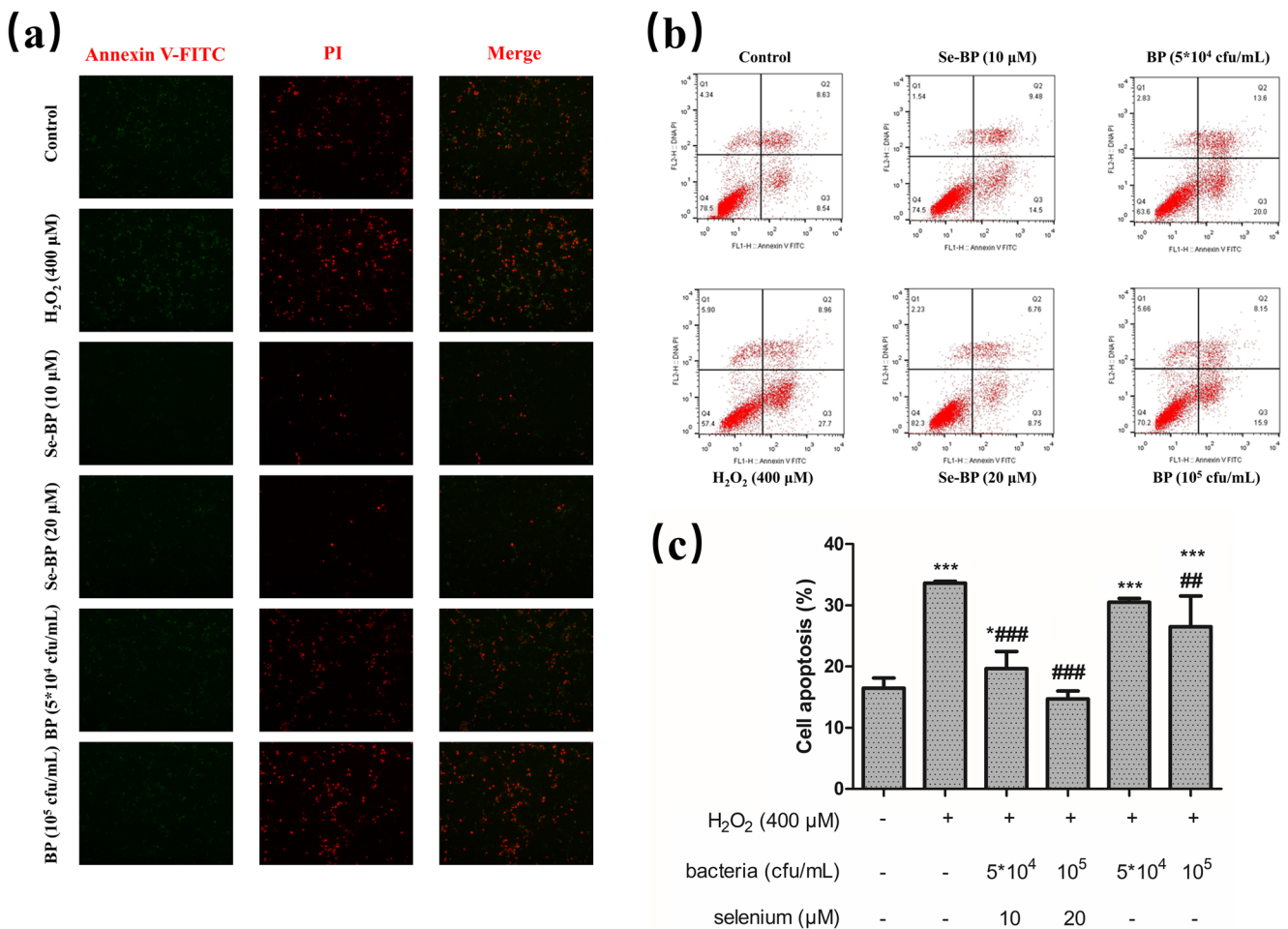
Fig. 4 Effects of Se-BP on H_2O_2-induced IPEC-J2 cell apoptosis. **a** Cells were pretreated with Se-BP (10 μM and 20 μM) or BP (5×10^4 cfu/mL and 10^5 cfu/mL) for 4 h and incubated with 400 μM H_2O_2 for 10 h, followed by staining with Annexin V-FITC/PI. Cell apoptosis was detected by fluorescence microscopy (**a**) and flow cytometry (**b**, **c**). The values are the means ± SDs (*$P < 0.05$, compared with untreated control cells; #$P < 0.05$, compared with the H_2O_2 group). Significant differences between the groups were analyzed with one-way ANOVA

IPEC-J2 cells, and preliminary experiments were conducted to determine the proper concentration of H_2O_2 for subsequent experiments.

On the one hand, many studies have demonstrated that different sources of selenium, such as selenium nanoparticles (Gangadoo et al. 2018; Song et al. 2017), sodium selenite, Se-enriched yeast, and DL-selenomethionine (Bakhshalinejad et al. 2017; Zamani Moghaddam et al. 2017), are necessary micronutrients with antioxidant, immunoregulatory, and anti-inflammatory effects on the gastrointestinal tract in both livestock and poultry. On the other hand, attention has been devoted to probiotics, which are defined as live microorganisms that confer health benefits on the host, especially antioxidant potential and anti-apoptosis ability, when administered in adequate amounts (Resta-Lenert et al. 2002). Combined mixtures called selenium-enriched probiotics, in which probiotics transform selenate and/or selenite to elemental selenium and/or organic selenium, have gradually attracted researchers' attention (Nancharaiah and Lens 2015).

Bacillus paralicheniformis SR14, a bacterial strain previously isolated by our group, synthesizes exopolysaccharide-capped elemental selenium, which was proven to protect IPEC-J2 cells against oxidative stress (Cheng et al. 2017). As we mentioned above, selenium-enriched probiotics have attracted widespread interest. However, the protective effect of selenium-enriched *Bacillus paralicheniformis* SR14 (Se-BP) against oxidative stress remains uncertain. Thus, in this study, we aimed to study the antioxidant effect of *Bacillus paralicheniformis* SR14 on IPEC-J2 cells.

First, we determined the ability of Se-BP to resist oxidative stress by assessing its free radical scavenging activity in vitro. Free radicals include four types: superoxide anion radicals, hydroxyl free radicals, DPPT, and ABTS·+. The superoxide anion radical, generated via the mitochondrial electron transport system, is considered an initial radical that generates other cell-damaging free radicals, such as hydrogen peroxide, hydroxyl radicals, and singlet oxygen, in living systems. The level of superoxide radicals is in dynamic balance under
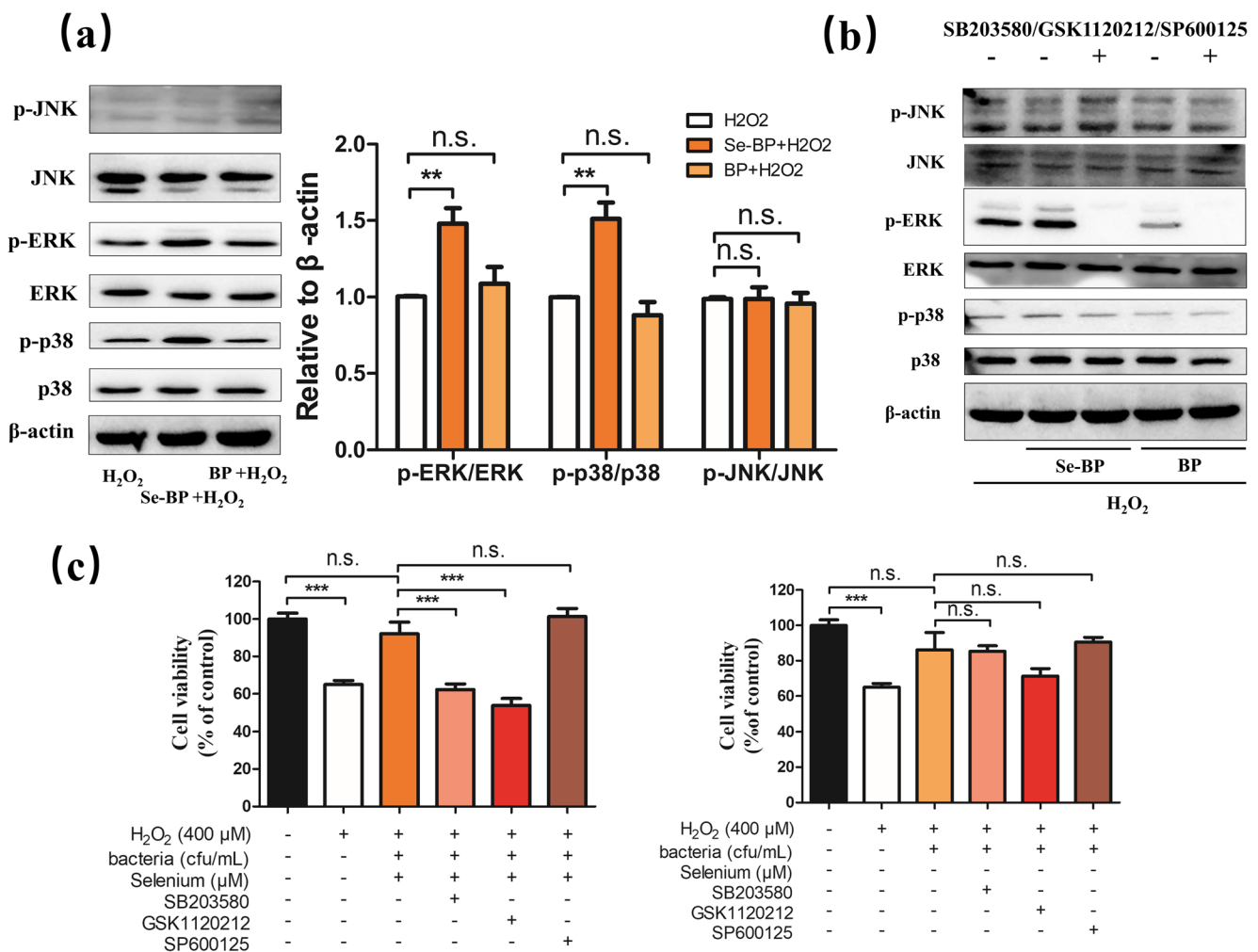
Fig. 5 Effects of Se-BP on MAPK signaling pathway activation in IPEC-J2 cells. **a** Cells were treated with Se-BP (20 μM) or BP (10^5 cfu/mL) for 4 h and incubated with 400 μM H_2O_2 for 10 h. Cell lysates were prepared and subjected to Western blotting to detect phospho-JNK, JNK, phospho-ERK, ERK, phospho-p38 MAPK, and p38 MAPK. The relative band densities of phosphorylated-JNK/ERK/p38 MAPK were normalized to those of JNK/ERK/p38 MAPK, respectively. **b**, **c** Cells were pretreated with inhibitors of p38 (SB203580, 25 μM), ERK (GSK1120212, 10 μM), and JNK (SP600125, 20 μM) for 1 h, followed by treatment with Se-BP

(20 μM) or BP (10^5 cfu/mL) for 4 h. Next, these cells were treated with 400 μM H_2O_2 for 10 h. Cell lysates were prepared and subjected to Western blotting to detect phospho-JNK, JNK, phosphor-ERK, ERK, phosphor-p38 MAPK, and p38 MAPK. The relative density of phosphorylated-JNK/ERK/p38 MAPK was relative to the level of JNK/ERK/p38 MAPK, respectively. Cell viability was measured via MTT assays. The values are the means ± SDs (*$P < 0.05$, compared with untreated control cells). Significant differences between the groups were analyzed with one-way ANOVA

normal conditions; however, under stress, excessive superoxide radicals harm the body (Blokhina et al. 2003). Hydroxyl free radicals, one type of ROS, are generated via a reaction between negative peroxide ions and hydrogen peroxide, which can degrade DNA and damage cells in vivo. Thus, the effective elimination of hydroxyl free radicals is beneficial to organisms (Liu et al. 2010). DPPH, a well-known free radical, has been widely considered an important indicator of the antioxidant capacity of biomaterials in vitro (Zhao et al. 2017). During the reaction, the color of the DPPH solution turns from purple to yellow. ABTS·+, which is generated by oxidants, is also inhibited by antioxidants, a property widely used to evaluate the total antioxidant capacity (Rezvanfar et al. 2013). As shown in Fig. 1, BP exhibited an ability to scavenge

superoxide radicals and DPPH, at a 100% hydroxyl scavenging rate, consistent with the findings of Gao's study (Gao et al. 2013). In contrast, Se-BP presented a greater ability than BP to scavenge all free radicals, especially ABTS radicals, which means that Se-BP demonstrated a higher antioxidant potential than BP. The above results provide a foundation for subsequent antioxidant research.

As selenium is an essential trace element whose safety threshold is very narrow (Nancharaiah and Lens 2015), we selected lower concentrations of 1–20 μM, which were not cytotoxic, as the initial selenium concentrations (Fig. S1 (b)) Thus, subsequent experiments were performed using Se-BP concentrations of ≤ 20 μM and BP concentrations of ≤ 10^5 cfu/mL for 4 h.

Second, we evaluated the protective effect of Se-BP on H_2O_2-induced oxidative stress. Cell viability and LDH release are important indicators of cell injury and integrity. The results showed that both Se-BP and BP had a tendency to attenuate the decline in H_2O_2-induced cell viability (Fig. 2a) and the increase in LDH release (Fig. 2b), consistent with the results of reported studies (Grompone et al. 2012).

ROS, which serve as critical signaling molecules in cell proliferation and survival, are important for cell homeostasis. However, excess ROS will surmount an effective antioxidant response (Ray et al. 2012). Treatment of cells with H_2O_2 causes excess ROS production and further results in oxidative stress. MDA is one of the final products of polyunsaturated fatty acid peroxidation in cells. Thus, MDA is commonly known as a reliable marker of oxidative stress and the antioxidant status (Gaweł et al. 2004). In this study, exposure of IPEC-J2 cells to H_2O_2 markedly induced cellular injuries as evidenced by the elevated levels of ROS and MDA. Pretreatment with Se-BP (10 μM and 20 μM) significantly inhibited this fatal damage, whereas pretreatment with BP (5 × 10^4 cfu/mL, 10^5 cfu/mL) only slowed this damage rather than completely preventing it. This inhibition of H_2O_2-induced ROS generation and MDA production by pretreatment with Se-BP may occur via a direct antioxidative mechanism through free radical scavenging activity, similar to the above results (Fig. 1).

Previous studies demonstrated that selenium and probiotics administered separately could effectively modulate the apoptosis of damaged cells (Bidkar et al. 2017; Resta-Lenert et al. 2002; Yan and Polk 2002). Flow cytometric analysis and Annexin V-FITC/PI assays further confirmed that increase in ROS levels led to cell death after infection and that Se-BP (10 μM and 20 μM) and BP (1 × 10^5 cfu/mL) effectively ameliorated H_2O_2-induced cell apoptosis (Fig. 4), consistent with the results of previous studies about functionalized selenium (Gao et al. 2014). Combining the results of the free radical scavenging activity, cell viability, LDH release, ROS generation, MDA production and cell apoptosis assays, we conclude that compared to BP pretreatment, Se-BP pretreatment significantly attenuated oxidative damage; these results were consistent with those of a study focused on selenium-enriched lactobacillus and lactobacillus (Patel et al. 2016).

To explore the possible mechanism underlying the antioxidant effects of Se-BP, we focused on cellular signal pathways. Cells need to be constantly aware of changes in the extracellular milieu to respond accordingly, so they have developed sophisticated mechanisms to receive signals, transmit the information, and orchestrate the appropriate responses. Signal transduction mechanisms rely heavily on posttranslational modifications of proteins, among which phosphorylation plays a major role. Current knowledge suggests that the kinases referred to as MAPKs seem to be involved in most signal transduction pathways (Raman et al. 2007). Studies have reported that MAPK is an important regulator of cell growth, survival, proliferation, inflammation, and immune reactions in response to oxidative stress (Rui et al. 2015). Our results showed that Se-BP but not BP can activate the ERK/ p38 MAPK signaling pathway to protect against oxidative injury induced by H_2O_2 (Fig. 5). However, the role of MAPKs is controversial; some studies have reported that different materials play antioxidant roles by activating of MAPKs (Borrás et al. 2005),whereas others have suggested that oxidative damage or stress contributes to the activation of MAPKs (Haddad and Land 2002; Nafees et al. 2014; Rui et al. 2015). Further work needs to be done to verify the exact effect of MAPKs under conditions of cellular oxidative damage.

In conclusion, Se-BP might play an important role in protecting cells from oxidative stress by activating the ERK/ p38 MAPK signaling pathway. Although a few studies have attempted to uncover the effect of selenium-enriched probiotics on the antioxidant status and immune function, these studies mainly focused on applications in pigs or rats, which lack the potential mechanisms demonstrated in cell models. Our study focused on the interaction between cellular oxidative damage and selenium-enriched probiotics, as well as the underlying mechanism. As we aim to identify safe and efficient dietary supplement of selenium-enriched probiotics, further investigations are needed in vivo in the future. Se-BP may indeed have broad applications in oxidative stress-related diseases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval The research performed did not involve human participants and/or animals.

References

Aw TY (1999) Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine. Am J Clin Nutr 70(4):557–565

Bakhshalinejad R, Akbari RMK, Zoidis E (2017) Effects of different dietary sources and levels of selenium supplements on growth performance, antioxidant status and immune parameters in Ross 308 broiler chickens. Br Poult Sci 59(1):81–91. https://doi.org/10.1080/00071668.2017.1380296

Balaban H, Naziroglu M, Demirci K, Ovey IS (2017) The protective role of selenium on scopolamine-induced memory impairment, oxidative stress, and apoptosis in aged rats: the involvement of TRPM2 and TRPV1 channels. Mol Neurobiol 54(4):2852–2868. https://doi.org/10.1007/s12035-016-9835-0

Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE (2014) Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev 94(2):329–354. https://doi.org/10.1152/physrev.00040.2012

Bhatti JS, Bhatti GK, Reddy PH (2017) Mitochondrial dysfunction and oxidative stress in metabolic disorders - a step towards mitochondria based therapeutic strategies. Bba-Mol Basis Dis 1863(5):1066–1077. https://doi.org/10.1016/j.bbadis.2016.11.010

Bidkar AP, Sanpui P, Ghosh SS (2017) Efficient induction of apoptosis in cancer cells by paclitaxel-loaded selenium nanoparticles. Nanomedicine 12(21):2641–2652. https://doi.org/10.2217/nnm-2017-0189

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot-London 91(2):179–194. https://doi.org/10.1093/aob/mcf118

Borrás C, Gambini J, Gómez-Cabrera MC, Sastre J, Pallardó FV, Mann GE, Viña J (2005) 17β-oestradiol up-regulates longevity-related, antioxidant enzyme expression via the ERK1 and ERK2[MAPK]/NFκB cascade. Aging Cell 4(3):113–118. https://doi.org/10.1111/j.1474-9726.2005.00151.x

Chen L, Pan DD, Zhou J, Jiang YZ (2005) Protective effect of selenium-enriched lactobacillus on CCl4-induced liver injury in mice and its possible mechanisms. World J Gastroenterol 11(37):5795–5800. https://doi.org/10.3748/wjg.v11.i37.5795

Cheng YZ, Xiao X, Li XX, Song DG, Lu ZQ, Wang FQ, Wang YZ (2017) Characterization, antioxidant property and cytoprotection of exopolysaccharide-capped elemental selenium particles synthesized by Bacillus paralicheniformis SR14. Carbohydr Polym 178:18–26. https://doi.org/10.1016/j.carbpol.2017.08.124

Danesi F, Malaguti M, Di NM, Maranesi M, Biagi PL, Bordoni A (2006) Counteraction of adriamycin-induced oxidative damage in rat heart by selenium dietary supplementation. J Agric Food Chem 54(4):1203–1208. https://doi.org/10.1021/jf0518002

Feng C, Zhang Y, Yang M, Lan M, Liu H, Huang B, Zhou Y (2017) Oxygen-sensing Nox4 generates genotoxic ROS to induce premature senescence of nucleus pulposus cells through MAPK and NF-κB pathways. Oxidative Med Cell Longev 2017(1):7426458. https://doi.org/10.1155/2017/7426458

Gan F, Ren F, Chen XX, Lv CH, Pan CL, Ye GP, Shi J, Shi XL, Zhou H, Shituleni SA, Huang KH (2013) Effects of Selenium-enriched probiotics on heat shock protein mRNA levels in piglet under heat stress conditions. J Agric Food Chem 61(10):2385–2391. https://doi.org/10.1021/jf300249j

Gangadoo S, Dinev I, Chapman J, Hughes RJ, Van TTH, Moore RJ, Stanley D (2018) Selenium nanoparticles in poultry feed modify gut microbiota and increase abundance of Faecalibacterium prausnitzii. Appl Microbiol Biotechnol 102(3):1455–1466. https://doi.org/10.1007/s00253-017-8688-4

Gao D, Gao Z, Zhu G (2013) Antioxidant effects of Lactobacillus plantarum via activation of transcription factor Nrf2. Food Funct 4(6):982–989. https://doi.org/10.1039/c3fo30316k

Gao FP, Yuan Q, Gao L, Cai PJ, Zhu HR, Liu R, Wang YL, Wei YT, Huang GD, Liang J, Gao XY (2014) Cytotoxicity and therapeutic effect of irinotecan combined with selenium nanoparticles. Biomaterials 35(31):8854–8866. https://doi.org/10.1016/j.biomaterials.2014.07.004

Gaweł S, Wardas M, Niedworok E, Wardas P (2004) Dialdehyd malonowy (MDA) jako wskaznik procesow peroksydacji lipidow w organizmie[Malondialdehyde (MDA) as alipid peroxidation marker]. Wiad Lek 57(9-10):453–455

Grompone G, Martorell P, Llopis S, Gonzalez N, Genoves S, Mulet AP, Fernandez-Calero T, Tiscornia I, Bollati-Fogolin M, Chambaud I, Foligne B, Montserrat A, Ramon D (2012) Anti-inflammatory Lactobacillus rhamnosus CNCM I-3690 strain protects against oxidative stress and increases lifespan in Caenorhabditis elegans. PLoS One 7(12):e52493. https://doi.org/10.1371/journal.pone.0052493

Guan MC, Tang WH, Xu Z, Sun J (2014) Effects of selenium-enriched protein from ganoderma lucidum on the levels of IL-1 beta and TNF-alpha, oxidative stress, and NF-kappa B activation in ovalbumin-induced asthmatic mice. Evid-Based Compl Alt 2014:182817. https://doi.org/10.1155/2014/182817

Haddad JJ, Land SC (2002) Redox/ROS regulation of lipopolysaccharide-induced mitogen-activated protein kinase (MAPK) activation and MAPK-mediated TNF-alpha biosynthesis. Brit J Pharmacol 135(2):520–536. https://doi.org/10.1038/sj.bjp.0704467

Hall RJ, Gupta PL (1969) The determination of very small amounts of selenium in plant samples. Analyst 94(1117):292. https://doi.org/10.1039/an9699400292

Hatfield DL, Tsuji PA, Carlson BA, Gladyshev VN (2014) Selenium and selenocysteine: roles in cancer, health, and development. Trends Biochem Sci 39(3):112–120. https://doi.org/10.1016/j.tibs.2013.12.007

Je JY, Lee DB (2015) Nelumbo nucifera leaves protect hydrogen peroxide-induced hepatic damage via antioxidant enzymes and HO-1/Nrf2 activation. Food Funct 6(6):1911–1918. https://doi.org/10.1039/c5fo00201j

Liu XN, Zhou B, Lin RS, Jia L, Deng P, Fan KM, Wang GY, Wang L, Zhang JJ (2010) Extraction and antioxidant activities of intracellular polysaccharide from Pleurotus sp mycelium. Int J Biol Macromol 47(2):116–119. https://doi.org/10.1016/j.ijbiomac.2010.05.012

Liu HM, Bian WX, Liu SX, Huang KX (2012) Selenium protects bone marrow stromal cells against hydrogen peroxide-induced inhibition of osteoblastic differentiation by suppressing oxidative stress and ERK signaling pathway. Biol Trace Elem Res 150(1-3):441–450. https://doi.org/10.1007/s12011-012-9488-4

Maggini S, Wintergerst ES, Beveridge S, Hornig DH (2007) Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Brit J Nutr 98 Suppl 1(S1):S29–S35. https://doi.org/10.1017/S0007114507832971

Mccready RG, Campbell JN, Payne JI (1966) Selenite reduction by Salmonella Heidelberg. Can J Microbiol 12(4):703–714. https://doi.org/10.1139/M66-097

Musa HH, Wu SL, Zhu CH, Seri HI, Zhu GQ (2009) The potential benefits of probiotics in animal production and health. J Anim Vet Adv 8(2):313–321

Nafees S, Rashid S, Ali N, Sultana S (2014) Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in wistar rats: role of NF kappa B/MAPK pathway. Free Radical Bio Med 76:S151–S151. https://doi.org/10.1016/j.freeradbiomed.2014.10.564

Nancharaiah YV, Lens PNL (2015) Ecology and biotechnology of selenium-respiring bacteria. Microbiol Mol Biol R 79(1):61–80. https://doi.org/10.1128/Mmbr.00037-14

Nathan C, Cunningham-Bussel A (2013) Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nat Rev Immunol 13(5):349–361. https://doi.org/10.1038/nri3423

Nido SA, Shituleni SA, Mengistu BM, Liu YH, Khan AZ, Gan F, Kumbhar S, Huang KH (2016) Effects of selenium-enriched probiotics on lipid metabolism, antioxidative status, histopathological lesions, and related gene expression in mice fed a high-fat diet. Biol Trace Elem Res 171(2):399–409. https://doi.org/10.1007/s12011-015-0552-8

Patel B, Kumar P, Banerjee R, Basu M, Pal A, Samanta M, Das S (2016) Lactobacillus acidophilus attenuates Aeromonas hydrophila induced cytotoxicity in catla thymus macrophages by modulating

oxidative stress and inflammation. Mol Immunol 75:69–83. https://doi.org/10.1016/j.molimm.2016.05.012

Pescuma M, Gomez-Gomez B, Perez-Corona T, Font G, Madrid Y, Mozzi F (2017) Food prospects of selenium enriched-*Lactobacillus acidophilus* CRL 636 and *Lactobacillus reuteri* CRL 1101. J Funct Foods 35:466–473. https://doi.org/10.1016/j.jff.2017.06.009

Raman M, Chen W, Cobb MH (2007) Differential regulation and properties of MAPKs. Oncogene 26(22):3100–3112. https://doi.org/10.1038/sj.onc.1210392

Ravn-Haren G, Bugel S, Krath BN, Hoac T, Stagsted J, Jorgensen K, Bresson JR, Larsen EH, Dragsted LO (2008) A short-term intervention trial with selenate, selenium-enriched yeast and selenium-enriched milk: effects on oxidative defence regulation. Brit J Nutr 99(4):883–892. https://doi.org/10.1017/S0007114507825153

Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24(5):981–990. https://doi.org/10.1016/j.cellsig.2012.01.008

Resta-Lenert SC, Valle AM, Barrett KE (2002) Probiotics prevent apoptosis of intestinal epithelial cells induced by enteroinvasive pathogens. Gastroenterology 122(4):A281–A281

Rezvanfar MA, Rezvanfar MA, Shahverdi AR, Ahmadi A, Baeeri M, Mohammadirad A, Abdollahi M (2013) Protection of cisplatin-induced spermatotoxicity, DNA damage and chromatin abnormality by selenium nano-particles. Toxicol Appl Pharmacol 266(3):356–365. https://doi.org/10.1016/j.taap.2012.11.025

Rui W, Guan L, Zhang F, Zhang W, Ding W (2015) PM2.5 -induced oxidative stress increases adhesion molecules expression in human endothelial cells through the ERK/AKT/NF- kappa B-dependent pathway. J Appl Toxicol 36(1):48–59. https://doi.org/10.1002/jat.3143

Song D, Cheng Y, Li X, Wang F, Lu Z, Xiao X, Wang Y (2017) Biogenic nanoselenium particles effectively attenuate oxidative stress-induced intestinal epithelial barrier injury by activating the Nrf2 antioxidant pathway. ACS Appl Mater Interfaces 9(17):14724–14740. https://doi.org/10.1021/acsami.7b03377

Xu J, Yang FM, An XX, Hu QH (2007) Anticarcinogenic activity of selenium-enriched green tea extracts *in vivo*. J Agric Food Chem 55(13):5349–5353. https://doi.org/10.1021/jf070568s

Yan F, Polk DB (2002) Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. J Biol Chem 277(52):50959–50965. https://doi.org/10.1074/jbc.M207050200

Yang JJ, Huang KH, Qin SY, Wu XS, Zhao ZP, Chen F (2009) Antibacterial action of selenium-enriched probiotics against pathogenic *Escherichia coli*. Dig Dis Sci 54(2):246–254. https://doi.org/10.1007/s10620-008-0361-4

Yazdi MH, Mahdavi M, Setayesh N, Esfandyar M, Shahverdi AR (2013) Selenium nanoparticle-enriched *Lactobacillus brevis* causes more efficient immune responses *in vivo* and reduces the liver metastasis in metastatic form of mouse breast cancer. Daru 21:33. https://doi.org/10.1186/2008-2231-21-33

Zamani Moghaddam AK, Mehraei Hamzekolaei MH, Khajali F, Hassanpour H (2017) Role of selenium from different sources in prevention of pulmonary arterial hypertension syndrome in broiler chickens. Biol Trace Elem Res 180(1):164–170. https://doi.org/10.1007/s12011-017-0993-3

Zhang HB, Chen TF, Jiang J, Wong YS, Yang F, Zheng WJ (2011) Selenium-Containing Allophycocyanin purified from selenium-enriched *spirulina platensis* attenuates AAPH-induced oxidative stress in human erythrocytes through inhibition of ROS generation. J Agric Food Chem 59(16):8683–8690. https://doi.org/10.1021/jf2019769

Zhao Y, Wang Y, Jiang ZT, Li R (2017) Screening and evaluation of active compounds in polyphenol mixtures by HPLC coupled with chemical methodology and its application. Food Chem 227:187–193. https://doi.org/10.1016/j.foodchem.2017.01.085

Zou Y, Wang J, Peng J, Wei HK (2016) Oregano essential oil induces SOD1 and GSH expression through Nrf2 activation and alleviates hydrogen peroxide-induced oxidative damage in IPEC-J2 cells. Oxidative Med Cell Longev 5987183:1–13. https://doi.org/10.1155/2016/5987183