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Effects of Polyphenols and Glucosinolates in Broccoli Extract on Human Gut Microorganisms Based on Simulation In Vitro

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Cite This: *ACS Omega* 2022, 7, 45096−45106

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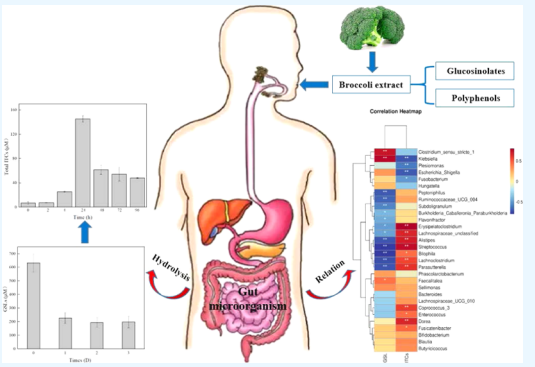
ABSTRACT: Broccoli extract mainly contains polyphenols and glucosinolates (GSLs). GSLs can be hydrolyzed by gut microorganisms into isothiocyanates (ITCs) and other active substances. These substances have anticancer, anti-inflammatory, antimicrobial, and atherosclerosis-reducing functions. In this study, a high concentration (2000 μmol/L GSLs and 24 μmol/L polyphenols) and a low concentration (83 μmol/L GSLs and 1 μmol/L polyphenols) of broccoli extract were prepared. Gut microorganisms from fresh human feces were cultured to simulate the gut environment in vitro. The GSL content decreased and the types and content of ITCs increased with broccoli extract hydrolysis through cyclic condensation and gas chromatography−mass spectrometry (GC-MS) analyses. Broccoli extract significantly increased probiotics and inhibited harmful bacteria through 16S rDNA sequencing. Based on phylum level analysis, *Firmicutes* and *Lachnospiraceae* increased significantly ($P < 0.05$). At the genus level, both high- and low-concentration groups significantly inhibited *Escherichia* and increased *Bilophila* and *Alistipes* ($P < 0.05$). The high-concentration group significantly increased *Bifidobacterium* ($P < 0.05$). The broccoli extract improved the richness of gut microorganisms and regulated their structure. The GSL hydrolysis was significantly correlated with *Bilophila, Lachnospiraceae, Alistipes, Bifidobacterium, Escherichia*, and *Streptococcus* ($P < 0.05$). These study findings provide a theoretical foundation for further exploring a probiotic mechanism of broccoli extract in the intestine.

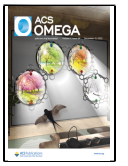


INTRODUCTION

Epidemiological studies have reported that regular consumption of cruciferous vegetables (e.g., broccoli, cabbage, kale, and Brussels sprouts) can reduce the risk of chronic diseases (e.g., atherosclerosis and cancer).[1,2] These beneficial properties are mainly attributable to the richness of natural antioxidant active substances present in cruciferous vegetables (e.g., polyphenols, glucosinolates (GSLs), flavonoids, anthocyanins, vitamin C, and carotenoids).[3,4] In particular, GSLs are unique sulfur-containing secondary metabolites of cruciferous vegetables and are chemically stable and biologically inert. GSLs can be hydrolyzed to isothiocyanates (ITCs) by endogenous myrosinase in plants. In intact crucifers, GSLs are localized to the vacuole, while myrosinase is present in a specific intracellular proteasome.[5] However, GSLs and myrosinase are released by crucifers during mechanical damage, infection, or pest infestation. Myrosinase hydrolyzes the released GSLs to generate one molecule of glucose and one aglycone. Aglycone is highly unstable and rearranges to form various hydrolytic products, mainly ITCs and nitriles.[6]

ITCs possess antioxidant, anti-inflammatory, and antibacterial properties.[7,8] Moreover, ITCs can induce the synthesis of type II detoxification enzymes and cancer cell apoptosis. They can also inhibit cancer cell proliferation and tumor cell invasion and metastasis *in vivo*.[9−12] The endogenous myrosinase in cruciferous vegetables is inactivated and cannot hydrolyze GSLs to ITCs during cooking.[13] Myrosinase loses approx 90% of its activity when cruciferous vegetables are sauted or heated at 60 °C for 3 min.[14] However, some reports have suggested that some gut microorganisms have the ability to hydrolyze GSLs to generate ITCs. Cordeiro et al.[15] and Elfoul et al.[16] demonstrated that sinigrin (a type of GSL) could be hydrolyzed to ITCs by *Bacteroides thetaiotaomicron* and *Escherichia* 0157:H7, respectively. Luang-In et al.[6] reported that *Enterococcus casseliflavus* CP1 and *Escherichia* VL8 could hydrolyze GSLs to generate ITCs and nitriles. Based on these results involving gut microbes, the research of gut microbiome involved in GSL hydrolysis has attracted considerable interest

Received: August 27, 2022
Accepted: November 25, 2022
Published: December 2, 2022



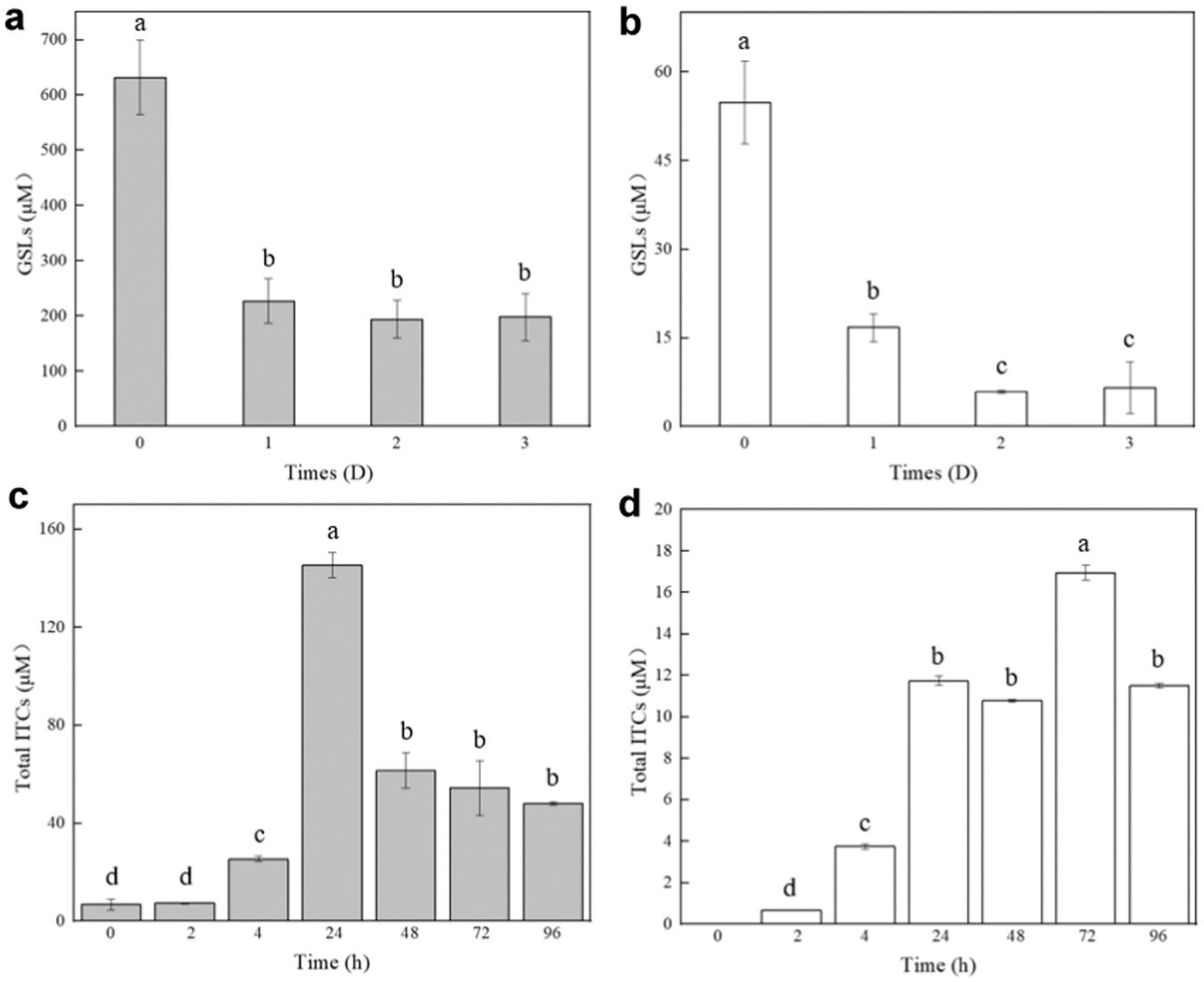


Figure 1. Changes of GSL content at high (a) and low (b) concentrations and ITC content at high (c) and low (d) concentrations with different culture times of microbiota. The data are represented as mean ± SD (n = 3). (a−d) Values with different superscripts are significantly different (P < 0.05).

because of the crucial role of these microbes in immunity and metabolism.[17]

The transformation of bioactive substances and their metabolites in food is essential for maintaining intestinal health in humans. They break indigestible dietary components in the intestine and provide nutrients and energy to the host. They promote metabolism and immune system of the host to maintain balance.[18] Many interactions occur between cruciferous metabolites and gut microorganisms. Therefore, it is critical to study the effects of broccoli extract on human gut microorganisms and broccoli extract hydrolyzed by gut microbes. In our previous experiments, the main active substances in the broccoli extract were mainly GSLs and polyphenols through the analysis of high-speed countercurrent chromatography.[19] In the present study, the broccoli extract was prepared and GSLs and polyphenols were measured. GSL hydrolysis by gut microflora and the effect of broccoli extract on these gut microbes were investigated through simulation in vitro, 16S rDNA sequencing and gas chromatography−mass spectrometry (GC-MS).

RESULTS

Broccoli Extract Hydrolyzed by Gut Microbes. Figure 1a,b presents the changes in GSL content at different times of culturing with gut microflora. The content of GSLs in the high-concentration group decreased significantly during 1-2 days (P < 0.05). In the low-concentration group, the content of GSLs decreased significantly during 1-2 days (P < 0.05) and almost could not be detected at 2-3 days. The changes in ITC content caused by gut microbes are shown in Figure 1c,d. The gut microbes began to hydrolyze the broccoli extract to ITCs at 2−4 h in the high-concentration group. The content of ITCs increased significantly at 4−24 h and decreased significantly at 24−48 h (P < 0.05). In the low-concentration group, ITC production occurred at 0−2 h. The ITC content increased significantly at 2−72 h (P < 0.05), whereas it decreased at 72−96 h.

Hydrolysis Products of Broccoli Extract. As shown in Table 1, the main hydrolysis products of broccoli extract in the high-concentration group were ITCs and nitriles. Sulforaphane nitrile and 2-methylbutyl isothiocyanate were detected in the H0 group. On day 1, 1-isothiocyanatobutane was the main

https://doi.org/10.1021/acsomega.2c05523
ACS Omega 2022, 7, 45096−45106

Table 1. Hydrolysis Products of Broccoli Extract by Gut Microorganisms

group	time (min)	hydrolysis products	content (μg/mL)
H0	14.97	sulforaphane nitrile	2.30
	18.10	2-methylbutyl isothiocyanate	7.10
H1	13.80	1-isothiocyanatobutane	4.10
	10.55	erucin nitrile	26.70
H2	13.80	1-isothiocyanatobutane	10.70
	16.42	isobutyl isothiocyanate	6.20
	23.22	4-methylphenyl isothiocyanate	154.70

hydrolysis product in the high-concentration group. The main hydrolysis products were mainly erucin nitrile, 1-isothiocyanatobutane, isobutyl isothiocyanate, and 4-methylphenyl isothiocyanate.

Cluster and Similarity Analysis on Effects of Broccoli Extract on Gut Microorganisms. The PCA, PCoA, NMDS, and UPGMA analyses are shown in Figure 2a−d, respectively. On days 1, 2, and 3, a large degree of dispersion was observed in the high-concentration group, which was significantly different from those in the other groups. Figure 3a−c shows the Anosim similarity analysis of microbes among the control, high-concentration group, and low-concentration group. The microorganisms of the high-concentration group, low-concentration group, and control were significantly different ($P < 0.05$).

Analysis of Microbial Diversity. Figure 4 illustrates the microbial diversity analysis at the phylum level. *Proteobacteria* in the high-concentration group decreased rapidly from 70.77 to 37.84% and then gradually increased to 54.21%. In the low-concentration group, *Proteobacteria* gradually decreased from 66.37 to 44.24%. *Firmicutes* in both high- and low-concentration groups increased from 10.12 and 8.94 to 33.28 and 38.77%, respectively. *Fusobacteria* in both high- and low-concentration groups decreased from 12.48 and 16.89 to 9.43 and 8.03%, respectively. *Bacteroidetes* in the high-concentration group increased from 6.29 to 8.31% and decreased to 1.28%, which was lower than the control. In the low-concentration group, *Bacteroidetes* increased from 7.35 to 10.82% and decreased to 8.34%.

The microbial diversity analyses at the genus level are shown in Figure 5a,b. *Escherichia* decreased rapidly from 59.41 to 36.05% and then increased to 52.49% in the high-concentration group. Similarly, it decreased from 61.92 to 36.07% in the low-concentration group. In both high- and low-concentration groups, *Fusobacterium* decreased from 12.48 and 16.89 to 9.42 and 8.03%, respectively. The high-concentration group of *Lachnoclostridium* gradually decreased from 3.13 to 1.24%. However, the low-concentration group exhibited a gradual increase in percentage from 2.77 to 8.93%. It was lower than that of the control (from 2.13 to 19.42%). *Bilophila* in the low-concentration group increased from 0.48 to 5.03% compared to the control. However, it did not change significantly in the high-concentration group. In the low-concentration group, *Alistipes* and *Burkholderia* increased from 0.41 and 0.68 to 2.89 and 1.21%, respectively. *Alistipes* decreased from 0.34 to 0.15% in the high-concentration group. Similarly, *Burkholderia* almost disappeared in the high-concentration group. *Blautia*, *Coprococcus*, *Phascolarctobacterium*, and *Bifidobacterium* only increased significantly in the high-concentration group from 0.07, 0.17, 2.22, and 0.01 to 1.37, 13.50, 3.52, and 1.47%, respectively. *Plesiomonas* decreased in the high- and low-concentration groups from 1.21 and 2.25 to 0.17 and 0.32%, respectively, which were lower than those in the control.

Figure 6 shows the microbial diversity analysis at the species level. In the high- and low-concentration groups, *Clostridium symbiosum* increased from 0.02 and 0.13 to 3.30 and 1.99%, respectively, and *Clostridium clostridioforme* increased from 0.11 and 0.18 to 3.42 and 1.51%, respectively. However, in the high-concentration group, *C. symbiosum* and *C. clostridioforme* decreased from 0.54 and 0.18 to 0.05 and 0.29%, respectively.

Bacterial Phenotype Analysis. As shown in Figure 7, the aerobic bacteria decreased and then increased in both high- and low-concentration groups, while the control exhibited a gradually increased trend (Figure 7a). Inversely, the anaerobic bacteria in the high- and low-concentration groups increased and then decreased (Figure 7b). The stability of the biofilm decreased and then increased in the high-concentration group. However, it gradually decreased in the low-concentration group and control (Figure 7c). The stress tolerance decreased and then increased gradually in the high-concentration group. In the low-concentration group and control, it decreased (Figure 7d). Potential pathogenic bacteria in the high- and low-concentration groups were lower than those in the control (Figure 7e).

Analysis of Correlation among GSLs, ITCs, and Microorganisms. Figure 8 displays the increase in ITC content with GSL hydrolysis. The hydrolysis of GSLs was significantly related to *Erysipelatoclostridium*, *Lachnospiraceae*, *Alistipes*, *Streptococcus*, *Bilophila*, *Parasutterella*, *Lachnoclostridium*, *Dorea*, *Coprococcus*, *Fusicatenibacter*, and *Enterococcus* ($P < 0.05$).

DISCUSSION

The content of GSLs in both high- and low-concentration groups decreased significantly at 1-2 days and then almost disappeared (Figure 1), indicating that the gut microorganisms effectively hydrolyzed GSLs. It is noticed that the ITC content in the high- and low-concentration groups was the maximum at 24 and 72 h, respectively. Then, the content of ITCs decreased gradually (Figure 1). This might be due to the self-protection mechanism exerted by microorganisms. The GSLs were hydrolyzed to ITCs and nitriles. The microorganisms tend to produce nitriles that are not harmful to them.[26,27] Luang-In et al.[6] reported that several gut microbes could produce sulfatase, which could remove the sulfate group from GSLs, thereby converting them to desulfurized GSLs and finally to nitriles. Gut microbes could also induce side-chain modification of GSLs. Narbad and Rossiter[28] demonstrated that some microbes were able to restore glucoraphanin to glucoerucin, which was then hydrolyzed to erucin nitrile. Another reason for the decreased ITCs is their poor stability and easy degradation.[29] The GC-MS result analysis of this study also showed that the GSL hydrolysis products contained nitriles (especially, erucin nitrile) in addition to ITCs, which was in accordance with the self-protective mechanism exerted by microbes.

The results of 16S rDNA sequencing showed that the high-concentration group had more significant effects on the structure and abundance of gut microorganisms than the low-concentration group compared with the control. It might be caused by the synergistic action of polyphenols and ITCs in broccoli extract. Our previous studies have shown that the antioxidant activity of broccoli extract was higher than that of

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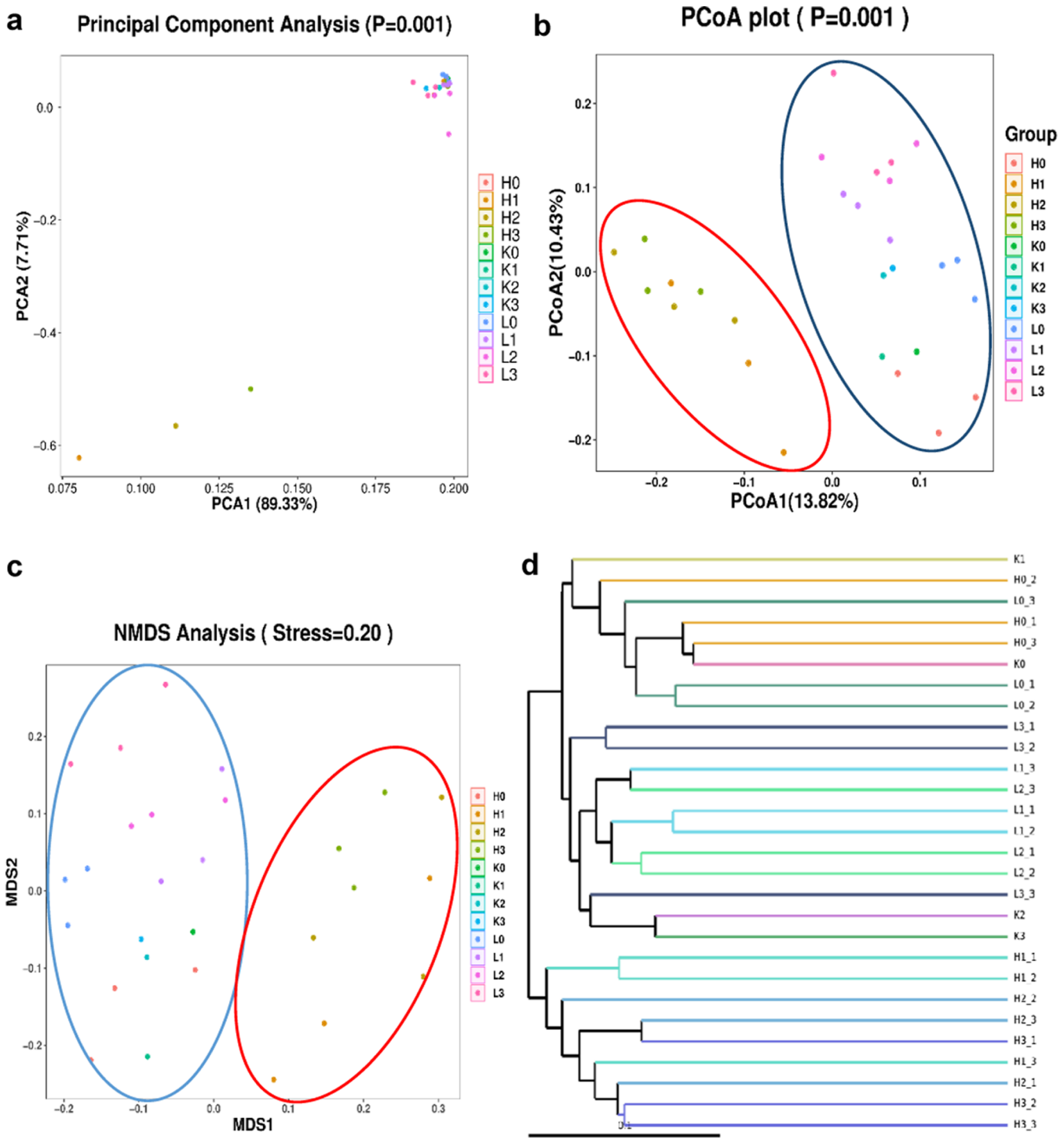

Figure 2. Analysis of PCA (a), PCoA (b), NMDS (c), and UPGMA (d). H: high concentration of the broccoli extract group; L: low concentration of the broccoli extract group; K: control. The data are represented as mean ± SD (n = 3).

the pure sulforaphane (a type of GSL derivative), which was due to the presence of polyphenols in the extract.[19] Polyphenols as functional substances can inhibit the growth of harmful microbes in the intestinal tract and reduce the toxicity induced by pathogenic bacteria. They can also provide metabolic substrates for gut microbes and exert their own characteristics.[30] Moreover, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were significantly changed in the high-concentration group. The relative abundance of *Bacteroidetes* decreased by 5.1% in the H. Tannock et al.[31] and Hughes et al.[32] have shown that the increased abundance of *Bacteroides* in the gut could lead to cancer. The high-concentration group significantly exhibited an increase in the relative abundance of beneficial bacteria (e.g., *Bifidobacterium*, *Blautia*, *Coprococcus*, and *Phascolarctobacterium*). The *Bifidobacterium* plays an important physiological role in enhancing immunity, improving gastrointestinal function, and resisting tumor.[33] *Coprococcus* can convert fructose to butyrate and lactate to propionate. Butyrate is a crucial energy substance for intestinal epithelial cells and has a necessary role in the development of intestinal

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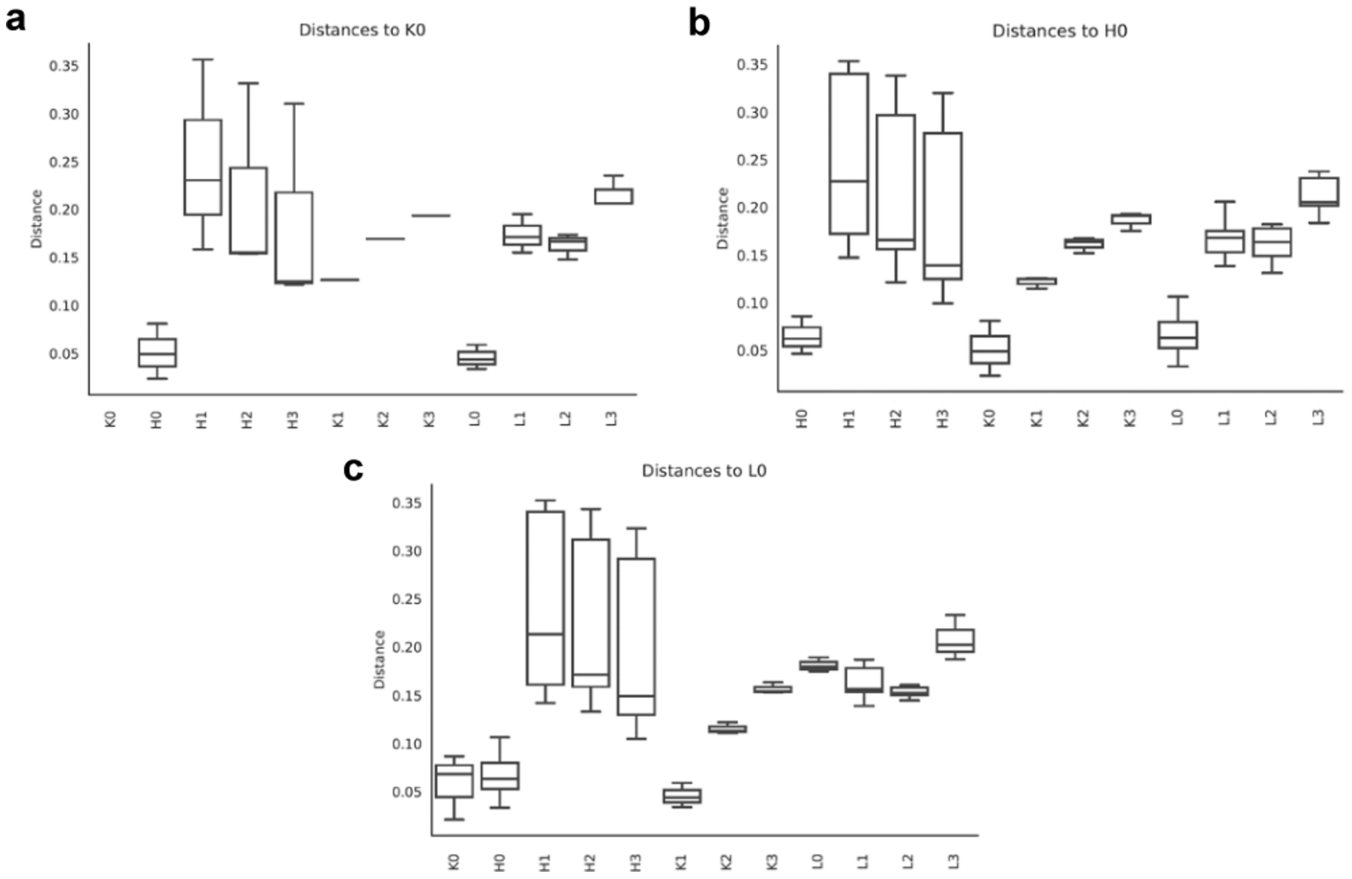

Figure 3. Analysis of Anosim similarity compared with K0 (a), H0 (b), and L0 (c). H: high concentration of the broccoli extract group; L: low concentration of the broccoli extract group; K: control. The data are represented as mean ± SD (n = 3).

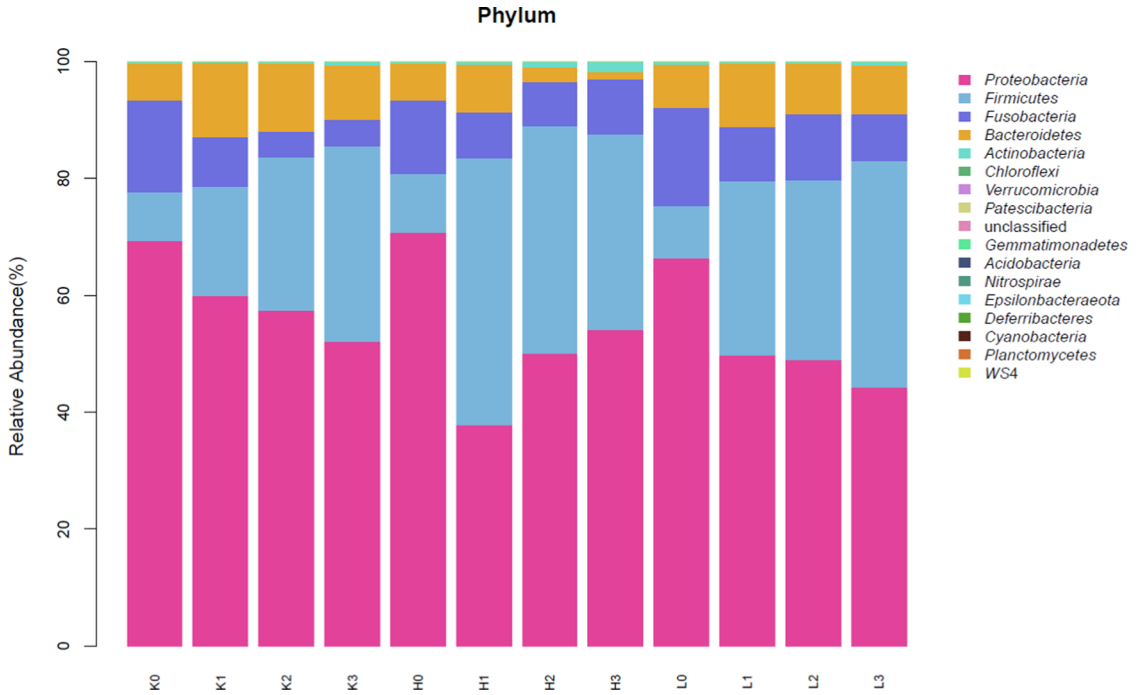

Figure 4. Relative abundance of microorganisms at the phylum level. H: high concentration of the broccoli extract group; L: low concentration of broccoli extract group; K: control. The data are represented as mean ± SD (n = 3).

epithelial cells.[34] Propionate has a positive role in blood pressure regulation.[35] The *Coprococcus* and *Faecalibacterium* are positively associated with higher quality of life indicators (QOLIs).[36] The *Blautia* can utilize complex carbohydrates to inhibit low-grade inflammation and alleviate intestinal barrier dysfunction.[37] The *Phascolarctobacterium* can effectively inhibit

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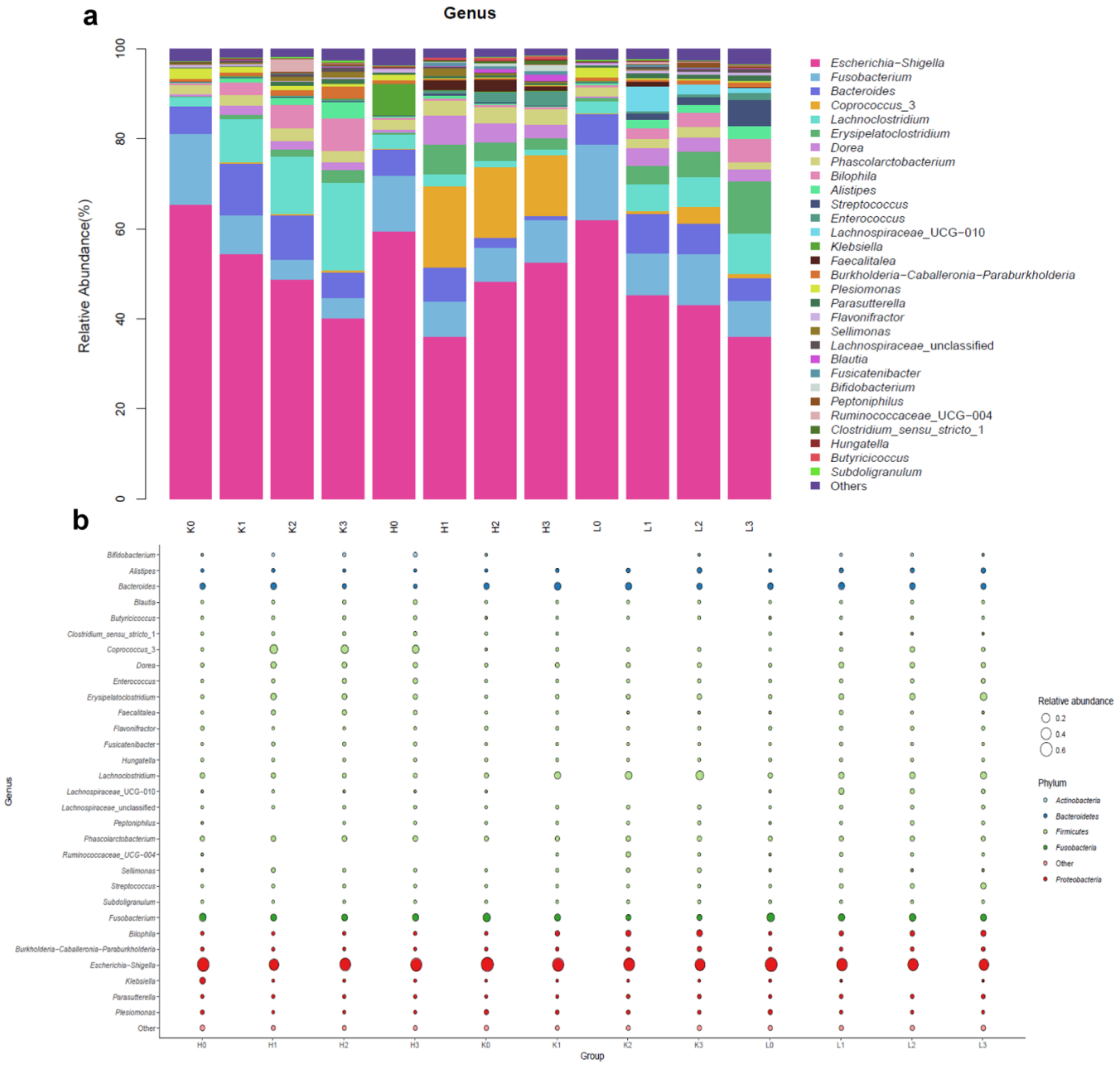

Figure 5. Relative abundance of microorganisms (a) and bubble plot analysis (b) at the genus level. H: high concentration of the broccoli extract group; L: low concentration of the broccoli extract group; K: control. The data are represented as mean ± SD (n = 3).

the growth of *Clostridioides* in the intestine by utilizing succinate produced by other intestinal bacteria and regulate the intestinal balance.[38] This might be one of the reasons for which the high-concentration group could significantly inhibit the growth of *C. symbiosum*. Xie et al.[39] found that *C. symbiosum* in colorectal adenoma (CRA), early colorectal cancer (CRC), and advanced CRC exhibited a trend of significant increase ($P < 0.05$). It outperformed other markers in early CRC prediction performance. Therefore, *C. symbiosum* is a biomarker useful for the noninvasive early detection of CRC. Combined with fecal immunochemistry (FIT) or carcinoembryonic antigen (CEA), *C. symbiosum* can improve the diagnostic ability.[39] It is indicated that broccoli extract can adjust the structure of gut microbes by adjusting the relative abundance ratio of key bacteria. It can also increase the beneficial bacteria and reduce the harmful bacteria.

Phenotype analysis revealed that the abundance of microbes in the high-concentration group, except potential pathogenic bacteria, decreased and then increased with the addition of the broccoli extract (Figure 7). It is indicated that broccoli extract had a great effect on the microorganisms. Regarding the correlation among GSLs, ITCs, and microorganisms, the beneficial bacteria *Bilophila*, *Lachnospiraceae*, and *Alistipes* had a crucial role in the hydrolysis of GSLs and production of ITCs (Figure 8). Kivenson and Giovannoni[40] reported that animal diet is the main source of trimethylamine (TMA). The gut microbes metabolized TMA to produce trimethylamine oxide (TMAO), which was associated with the cardiovascular disease. Interestingly, *Bilophila* could metabolize TMA but not produce TMAO, indicating the decreased risk of animal diet-induced cardiovascular disease. In the antiradiation experiments of mice, Guo et al.[41] found that "elite mice"

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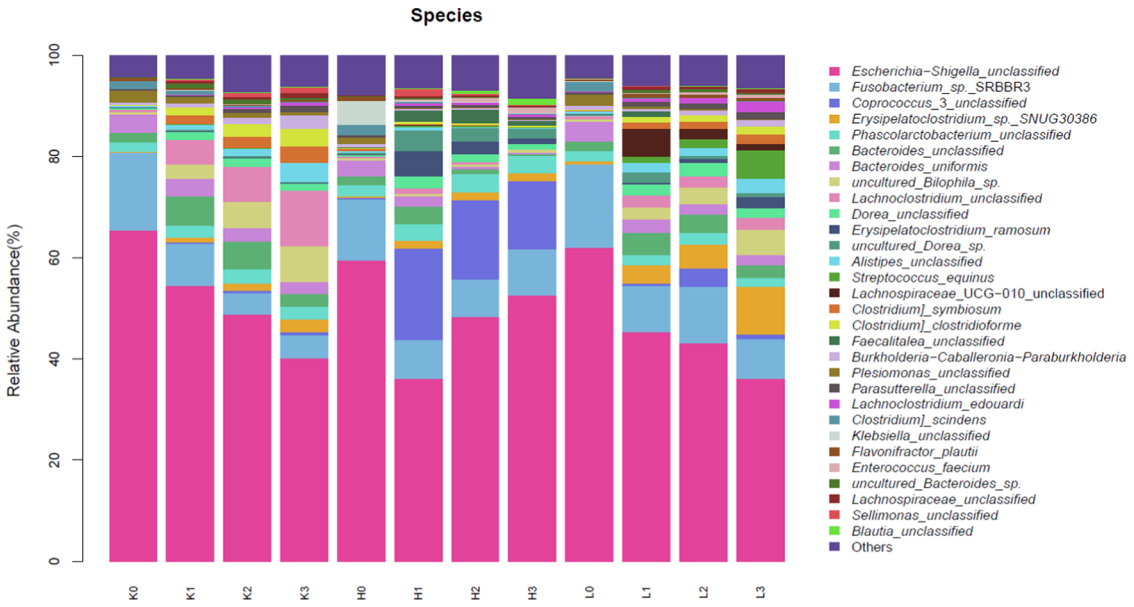

Figure 6. Relative abundance of microorganisms at the species level. H: high concentration of the broccoli extract group; L: low concentration of the broccoli extract group; K: control. The data are represented as mean ± SD (n = 3).

possessed a large number of *Lachnospiraceae*, which can promote hematopoiesis and renovate the intestinal tract by producing a large amount of short-chain fatty acids and metabolites of tryptophan metabolic pathways. It can prevent body from radiation-induced damage and death. Liu et al.[42] reported that *Alistipes* and *Oscillibacter* could reduce triglyceride levels and inhibit obesity and hyperlipidemia. Figure 5 shows that the broccoli extract could increase the beneficial bacteria (e.g., *Bilophila*, *Alistipes*, and *Bifidobacterium*), whereas the harmful bacteria growth (e.g., *Lachnoclostridium* and *Enterococcus*) is consistent with the phenotypic analysis of potential pathogenic bacteria shown in Figure 8. It indicated that intestinal beneficial bacteria (e.g., *Bilophila*, *Alistipes*, and *Bifidobacterium*) were necessary for promoting the probiotic effects of GSLs and ITCs in the intestine. This study demonstrated that the gut microorganisms could hydrolyze GSLs to generate ITCs and nitriles. Broccoli extract could improve the richness of gut microorganisms and regulate their structure. The high-concentration group had a more significant effect than the low-concentration group, which not only increased the probiotics (e.g., *Bilophila*, *Alistipes*, and *Bifidobacterium*) but also inhibited harmful bacteria (e.g., *Lachnoclostridium* and *Enterococcus*). The study results provided a theoretical foundation for further exploration of the effect of broccoli extract on gut microorganisms and the probiotic mechanism of the extract in the intestine.

EXPERIMENTAL PROCEDURE

Materials. Broccoli seeds were purchased from Qingfengyingke Seed Co., Ltd. (Guangdong, China). Ethyl acetate, petroleum ether, methanol, K_2HPO_4, and KH_2PO_4 were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Peptone, yeast extract, tryptone, cysteine hydrochloride, bile salt, heme, NaCl, Tween 80, $CaCl_2{\cdot}6H_2O$, $MgSO_2$, and $NaHCO_3$ were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Allyl isothiocyanate (analytical grade) and 1,4-benzenedithiol (>95%) were purchased from Sigma-Aldrich (MO). Nitrogen (≥ 99.999%) and mixed gas were obtained from Jingong Gas Co., Ltd. (Hangzhou, China).

Gut Microorganisms Based on Simulation In Vitro. The culture medium for gut microbes contained peptone (3.0 g/L), yeast extract (4.5 g/L), tryptone (3.0 g/L), cysteine hydrochloride (0.8 g/L), bile salt (0.4 g/L), heme (0.05 g/L), NaCl (4.5 g/L), Tween 80 (1.0 mL/L), KH_2PO_4 (0.04 g/L), K_2HPO_4 (0.04 g/L), $CaCl_2{\cdot}6H_2O$ (0.01 g/L), $MgSO_4{\cdot}7H_2O$ (0.01 g/L), and $NaHCO_3$ (2.0 g/L). The feces of three healthy young adults (age: 20−25 years), who did not use antibiotics within 6 months before the study were collected as gut microorganism samples. Participants signed an informed consent form and volunteered to provide feces as the gut microorganism samples. After the donors collected the feces using a disposable sterile sampler, the fecal samples were rapidly mixed and then immediately transferred to an anaerobic box. The feces (6 g) were added to 40 mL of sterile PBS (pH 6.8) buffer and filtered with multilayer sterile gauze. The supernatant was immediately placed in an anaerobic environment. The culture medium (45 mL) and supernatant solution (5 mL) were mixed and cultured in an anaerobic box (5% CO_2, 10% H_2, and 85% N_2) at 37 °C for 24 h to activate gut microorganisms. This sample was the control and named as K0. The broccoli extract (2000 μmol/L GSLs in the high-concentration group and 83 μmol/L GSLs in the low-concentration group) was added to a flask after activation. High- and low-concentration groups were regarded as H and L, respectively. Samples (5 mL) of the high-concentration group, low-concentration group, and control were collected every 24 h for 4 days, respectively. Three samples were collected at each time point for the high-concentration group, low-concentration group, and control. The experiment assays were conducted as three replicates. Samples collected from the high-concentration group on days 0, 1, 2, and 3 were H0, H1, H2, and H3, respectively. Samples collected from the low-concentration group on in days 0, 1, 2, and 3 were L0, L1, L2, and L3, respectively. Samples collected from the control on days 0, 1, 2, and 3 were K0, K1, K2, and K3, respectively. The samples were stored at −80 °C for subsequent experiment analysis.

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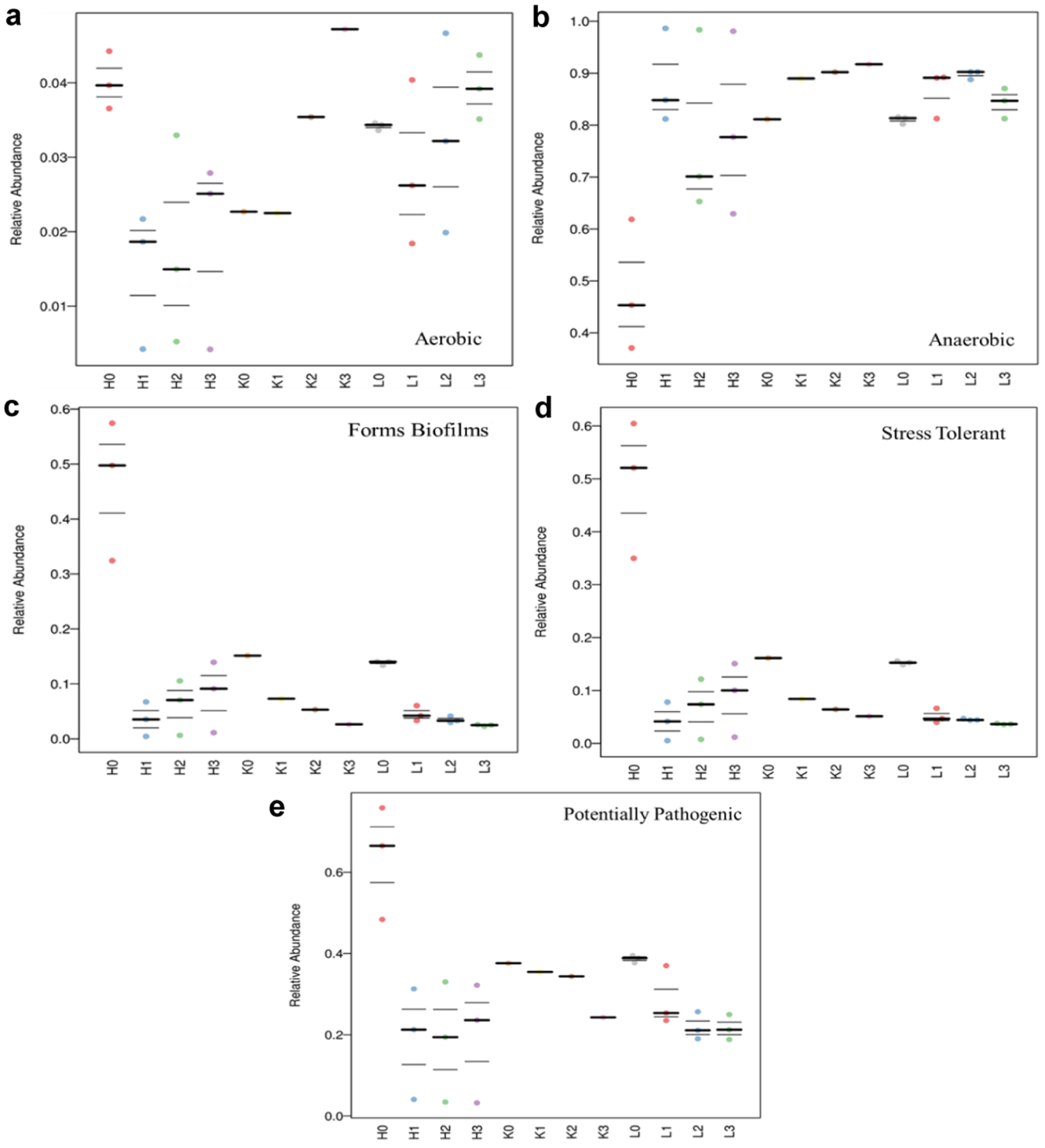

Figure 7. Analysis of bacterial phenotype in aerobic (a), anaerobic (b), forms biofilms (c), stress tolerant (d), and potentially pathogenic (e) conditions. H: high concentration of the broccoli extract group; L: low concentration of the broccoli extract group; K: control. The data are represented as mean ± SD (n = 3).

Broccoli Extract Preparation. The broccoli extract was prepared according to the method of Sarvan et al.[20] with some modifications. Broccoli (50 g) was incubated at 100 °C for 5 h to inactivate the myrosinase. Then, the broccoli was defatted and mixed with petroleum ether (500 mL) for 3 h and dried for 24 h. The defatted broccoli powder was added to 70% methanol (70 mL) with glass beads and extracted using a Soxhlet extractor to obtain crude broccoli extract. The crude broccoli extract was purified using HP-20 resin (35 mm × 150 mm, Anhui Samsung Resin Technology Co., Ltd. Anhui, China). The method is based on Ji et al.[21] with some modifications. Briefly, the resin was washed with 4% NaOH and 5% HCl solution and deionized water. The sample (100 mL) was injected into an adsorption column and eluted with deionized water at a flow rate of 4.8 mL/min. The eluate was

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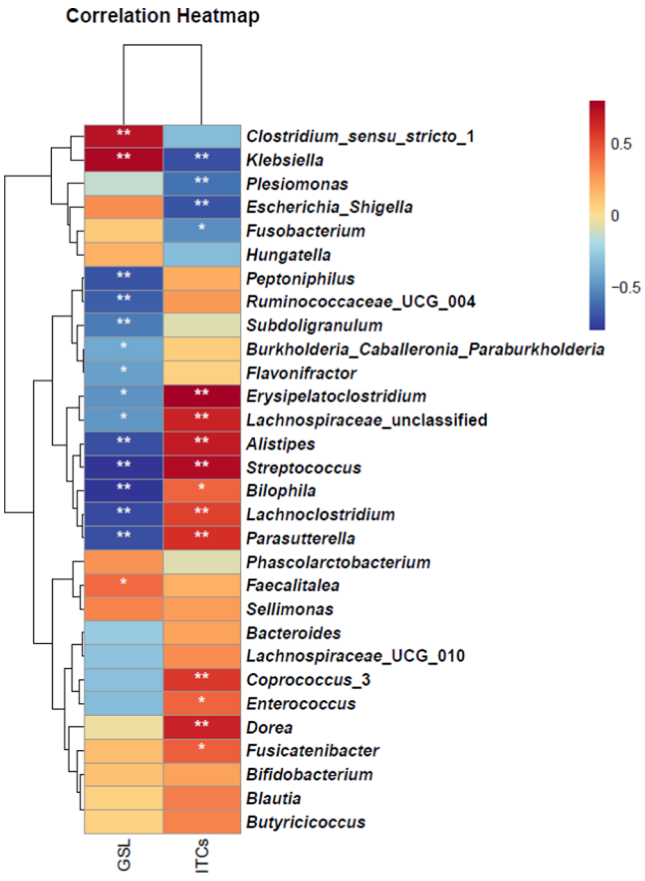

Figure 8. Correlation among GSLs, ITCs, and microorganisms at the genus level. *P < 0.05 and **P < 0.01 compared with the control.

collected, concentrated, fixed to 10 mL with deionized water and maintained at −80 °C for subsequent experiments.

ITC Quantification. The content of ITC was measured through a cyclocondensation method according to Wu et al.[22] with some modifications. A total of 200 μL of sample/standard was mixed with 300 μL of 20 mM potassium phosphate buffer (pH 8.5) and 400 μL of 10 mM 1,4-benzenedithiol. This mixture was incubated at 65 °C for 2 h. After the reaction, the sample was stored overnight and protected from light and centrifuged at 16,000g for 5 min. The supernatant was filtered through a microfiltration membrane (0.22 μm). The supernatant with cyclocondensation was analyzed using a Waters e2695 HPLC system (MA) equipped with a Waters 2489 detector.[23] The liquid-phase analysis was used with 80% methanol and 20% water used for fluidity, 1.0 mL/min as flow rate, 10 μL as injection volume, and 365 nm as detection wavelength. The chromatographic separation was carried out through a Wonda Cract ODS-2 column (4.6 mm × 250 mm i.d., 5 μm) (Shimadzu, Japan). The standard curve was established using different ITC standard concentrations for quantifying the ITC concentration of samples.

GSL Hydrolysis Level. The aforementioned broccoli extract or microorganism solution was centrifuged at 16,000g for 5 min. The supernatant (150 μL) was added to myrosinase and 450 μL of phosphate buffer (pH 7.0) for reaction for 4 h. The reaction solution was added to 1050 μL of ethyl acetate for extraction. The ethyl acetate phase was taken and dissolved in methanol after drying with nitrogen. The ITC content in broccoli extract and microorganism solution was measured and labeled as A_1 and A_0, respectively. The hydrolysis level of GSLs caused by gut microbes was calculated as follows: $G = A_1 - A_0$.

Polyphenol Content Measurement. The polyphenol content in broccoli extract was determined by the Folin phenol method.[24] Briefly, standard solutions of 0−50 μg/mL of gallic acid were prepared. The sample (1 mL) was mixed with 5 mL of 10% Folin phenol reagent and reacted for 4 min. Then, 4 mL of 7.5% Na_2CO_3 was added to the mixture and diluted to 10 mL for 2 h. The absorbance was measured at 760 nm. The content of polyphenols in broccoli extract was determined by a standard curve.

Analysis for Hydrolysis Products of Broccoli Extract. Hydrolysis products of broccoli extract caused by gut microorganisms were analyzed by GC-MS according to the method of Wu et al.[22] with some modifications. It was an ultrainserted capillary column (Hp-5MS, 30 m × 0.25 m i.d., 0.25 μm). The injection volume was 1 μL, and the vaporization chamber temperature was 300 °C. The program of the column temperature was set as follows: 50 °C for 2 min, 10 °C/min to 190 °C and 20 °C/min to 300 °C for 5 min. The carrier gas was helium in ultrahigh-purity grade with a 10:1 split ratio. The mass spectrometry conditions are as follows: the interface temperature is 220 °C, the ionization mode is EI, the ionization energy is 70 eV, and the mass range is 35500 amu. The cyclohexanone (0.24 mg/mL) mixed with the sample in an equal ratio was taken as the internal standard.

16S rDNA Sequencing Analysis. Every 1 mL sample (description in the "Gut Microorganisms Based on Simulation In Vitro" Section) of gut microbes with broccoli extract at days 0, 1, 2, and 3 for the control, low-concentration group, and high-concentration groups was selected and used to isolate DNA with an AxyPrep Bacterial Genomic DNA Miniprep Kit (Corning, New York).[25] The obtained nucleic acid samples were measured by an ultrafine spectrophotometer. The extracted genomic DNA was electrophoresed with 1% agarose gel electrophoresis for detecting integrity. The 16S rDNA sequencing and analysis were carried out through Lianchuan Biotechnology Co., Ltd. Hangzhou, China. After quality filtration to remove chimerical sequences, the resulting sequences were analyzed with an operational taxonomic unit.

Statistical Analysis. The experiment assays were conducted as three replicates. The results were statistically analyzed with SPSS 22.0. The data were represented as mean ± standard deviation (n = 3). Differences were considered significant at p < 0.05.

■ ASSOCIATED CONTENT

Data Availability Statement

The 16S rDNA gene sequencing dataset generated in this study is stored in the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/), and the project no. is PRJNA834976. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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https://doi.org/10.1021/acsomega.2c05523
ACS Omega 2022, 7, 45096−45106

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Author Contributions

Z.Y. and W.Y.F. conceived and designed research. J.C.M. and H.S.S. conducted experiments. S.J., S.X.J., N.S.Z., B.M.J., W.Y.F., and Z.Y. contributed new reagents or analytical tools. Z.Y. and W.Y.F. analyzed data. Z.Y. and W.Y.F. wrote the manuscript. All authors read and approved the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Key Research and Development Plan of Zhejiang Province (2019C02064), Natural Science Foundation of Zhejiang Province (LY16C200005), and Scientific Research Foundation of Zhejiang University of Science and Technology (F701103K07).

■ ABBREVIATIONS USED

GSLs, glucosinolates; ITCs, isothiocyanates; QOLI, quality of life indicator; CRA, colorectal adenoma; CRC, colorectal cancer; FIT, fecal immunochemistry; CEA, carcinoembryonic antigen; TMA, trimethylamine; TMAO, trimethylamine oxide

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https://doi.org/10.1021/acsomega.2c05523
ACS Omega 2022, 7, 45096−45106