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Effect of soil microbial feeding on gut microbiome and cadmium toxicity in *Caenorhabditis elegans*

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ABSTRACT

Microbial community of an organism plays an important role on its fitness, including stress responses. In this study, we investigated the effect of the culturable subset of soil microbial community (SMB) on the stress response of the soil nematode *Caenorhabditis elegans*, upon exposure to one of the major soil contaminants, cadmium (Cd). Life history traits and the stress responses to Cd exposure were compared between SMB- and *Escherichia coli* strain OP50-fed worms. SMB-fed worms showed higher reproduction rates and longer lifespans. Also, the SMB-fed worms showed more tolerant response to Cd exposure. Gene expression profiling suggested that the chemical stress and immune response of worms were boosted upon SMB feeding. Finally, we investigated *C. elegans* gut microbial communities in the presence and absence of Cd in OP50- and SMB-fed *C. elegans*. In the OP50-fed worms, changes in microbial community by Cd exposure was severe, whereas in the SMB-fed worms, it was comparatively weak. Our results suggest that the SMB affects the response of *C. elegans* to Cd exposure and highlight the importance of the gut microbiome in host stress response.

1. Introduction

In recent years, there have been numerous studies on the interaction between an organism (host) and the microorganisms living in its body (Morowitz et al., 2011; Claus and Jonathan, 2013; Zhang et al., 2015). These studies have demonstrated the considerable variety of effects that the microbiome can have on the host. In humans, changes to gut microbial communities, known as dysbiosis, can be an important cause of diseases such as allergies, diabetes, Crohn's disease, colorectal cancer, and even autism (Qin et al., 2012; Russell and Finlay, 2012; Nakatsu et al., 2015). Besides host-microbiome interaction, several studies have demonstrated that the gut microbiome can modulate the response of the host to chemical exposure. Relationships between the gut microbiome and chemicals can arise due to the microbiome metabolizing and/or biotransforming chemicals and thus changing its own composition (Kang et al., 2013). The gut microbiome can biologically transform chemicals via various biochemical reactions, such as reduction, hydrolysis, succinate group removal, dehydroxylation, acetylation, deacetylation, N-oxide bond cleavage, proteolysis, denitration, deconjugation, thiazole ring opening, deglycosylation, and demethylation (Sousa et al., 2008; Jeong et al., 2013; Claus et al., 2016). Thus, the gut microbiome can be a crucial mediator of chemical toxicity. The immune system is the first line of defense, playing a key role in host-microbiome interactions as well as in normal homeostasis and general stress responses. Furthermore, the immune system can be a target of xenobiotic-induced toxicity (Kim et al., 2018). Although much is known about the role of the immune system in host-microbiome interactions, little is known about how these interactions alter the host response to subsequent chemical exposure.

The soil nematode *Caenorhabditis elegans* is one of the most popular model organisms for genetics and developmental biology, however, the use of *C. elegans* in toxicology has also gained popularity in the past decade (Roh et al., 2009a; Hunt, 2017). *C. elegans* possesses several attributes that make it particularly useful for toxicology: a short reproductive life cycle, a large number of offsprings, and ease of maintenance. These attributes make it feasible to perform systematic investigation to predict toxicity. Being a bacterivore, *C. elegans* is also an attractive model for studying microbiome (Zhang et al., 2017). The *C. elegans* microenvironment can be artificially created using bacterivore features and bleaching eggs (Berg et al., 2016; Samuel et al., 2016). Genetically identical populations can also be produced, as *C. elegans* is a hermaphrodite. It is traditionally harvested in the presence of *Escherichia coli* strain OP50 in the lab culture condition (Brenner, 1974). The natural habitat of *C. elegans* is the vast microbiome pool found in decaying plant material in the soil (Félix and Braendle, 2010), however, the interactions between nematodes and other microorganisms have not been well studied.

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In this study, we aimed to investigate the effect of the culturable subset of Soil MicroBial community (SMB) on the response to chemical exposure in *C. elegans*, with a special focus on the role of the gut microbiome in host defense mechanisms. In our previous study, we studied the interaction between microorganisms and chemical toxicity using *C. elegans* pathogen infection model (Kim et al., 2018), in which cadmium (Cd) induced a sensitized response to the pathogen infection. To compare the effect of a single pathogen and the SMB on chemical toxicity responses, here also we used Cd, one of the major soil contaminants, as a model chemical. We first confirmed the effects of the SMB that colonized the gut microbiota on host life history traits, by comparing fitness of worms fed with OP50. We then investigated how responses to Cd exposure differed between the OP50- and SMB-fed worms using reproduction as an endpoint. After we identified the tolerant response in SMB-fed worms, we conducted gene expression analysis using a panel of immune and xenobiotic response genes to understand its mechanism. Finally, to understand how the gut microbiota is affected by Cd exposure, microbial community analysis was performed in the presence and absence of Cd in OP50- and SMB-fed worms.

2. Materials and methods

2.1. C. elegans growth and chemical

C. elegans strains were cultured at 20 °C on nematode growth medium (NGM) plates seeded with *E. coli* strain OP50 as described previously (Brenner, 1974). To synchronize their development stage, a number of gravid adults were rinsed with 10% hypochlorite solution to isolate the eggs, which were used in all subsequent experiments. The N2 Bristol strain, obtained from the Caenorhabditis Genetics Center (CGC), was used as the wild-type strain. Analytical-grade cadmium chloride was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Isolation and culture of soil microbial community

The soil microbial community was isolated from soil at an organic citrus farm (no chemical fertilizers or pesticides were used) in Pyoseonmyeon, Seogwipo-si, Jeju Island, Korea (33.341931, 126.779330). The soil was suspended in M9 media and centrifuged at 1800 rpm for 1 min. The supernatant was then carefully collected. After diluting the soil 10 to 10,000 times, the cells were seeded in a semi-NGM medium supplemented with yeast extract and incubated overnight in a shaking incubator at 37 °C. Bacterial colonies were collected and cultured again in Luria Bertani Broth. Consequently, only bacterial colonies that could grow in semi-NGM were obtained.

2.3. Microbial community analysis

2.3.1. Sampling

The SMB cultured on semi-NGM and worms fed with OP50 or SMB were sampled for microbial community analysis. The SMB cultured on semi-NGM was obtained by sub-culturing bacterial colonies from the soil extract plated on semi-NGM. Worms were collected after growing them from the L1 to the young adult stage in OP50- or SMB-seeded media. The worms were then washed with M9 media six times to remove any bacteria present on the surface (Berg et al., 2016; Samuel et al., 2016). The final washing solution was incubated in LB to confirm that all bacteria had been removed.

2.3.2. DNA isolation

For microbial community analysis, DNA was extracted from each sample (i.e. SMB, *C. elegans* fed with OP50 ± Cd and *C. elegans* fed with SMB ± Cd) using a FastDNA SPIN Kit for Soil (no. 116560200; MP Biomedicals, Solon, OH), according to the manufacturer's guidelines. The final volume of extracted DNA was 50 µl.

2.3.3. PCR amplification and illumina sequencing

To obtain a higher amount of bacterial DNA, PCR was carried out using the following primers targeting the V3–V4 region of the 16S rRNA gene (Fadrosh et al., 2014), the Nextera consensus - Sequencing adaptor - Target sequence, 341 F 5′-TCGTCGGCAGCGTC-AGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3′, 805 R 5′-GTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC -3′). The bacterial 16S rRNA was amplified under the following conditions: initial denaturation at 95 °C for 3 min, 25 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, and a final elongation at 72 °C for 5 min. To attach a barcode to the amplified DNA, secondary amplification was carried out. The barcodes used were as follows: Illumina NexTera barcode i5 forward primer (5′-AATGATACGGCGACCACCGAGATCTACAC-XXXXXXXX-TCGTCGGCAG CGTC-3′, X indicates the barcode region) and i7 reverse primer (5′-CAAGCAGAAGACGGCATACGAGAT-XXXXXXX-GTCTCGTGGGCTCGG-3′). The secondary amplification conditions were the same as those used for the primary amplification, except that the number of amplification cycles was set to 8. The mixed amplicons were then pooled and sequencing was conducted at Chunlab, Inc. (Seoul, Korea) using an Illumina MiSeq Sequencing system (Illumina, San Diego, CA, USA).

2.3.4. MiSeq pipeline method

Raw reads were quality checked and low quality (< Q25) reads were filtered using the Trimmomatic 0.321 software (Bolger et al., 2014). After passing the quality check, the paired-end sequence (250 bp) data were merged using PANDASeq2 software (Masella et al., 2012). Primers were then trimmed at a similarity cut off of 0.8. Mothur's pre-clustering program (3) was used to merge the resulting sequences and to extract unique sequences, allowing up to 2 differences between them. This process removed noise from the sequences. Of all the sequences that passed this process, 20,000 reads were randomly chosen and confirmed as chimera reads with a best hit similarity rate below 97% using UCHIME algorithm and the 16S database in the EzBioCloud (Edgar et al., 2011). The EzTaxon database was used to assign taxonomy using BLAST 2.2.224 and pairwise alignment to calculate sequence similarity (Myers and Miller, 1988; Altschul et al., 1990). Sequences with less than a 97% best hit similarity rate were detected using Uchime6 and the non-chimeric 16S rRNA database from EzTaxon.

2.4. C. elegans lifespan assay

A synchronized population of L1 worms were transferred to semi-NGM plates with different diets and allowed to develop into young adults. Approximately 90 young adult worms were divided on to three freshly seeded plates supplemented with 5-fluorodeoxyuridine (FuDR) at a final concentration of 0.1 mg/mL. FudR acts to reduce egg production in worms and prevent egg hatching without affecting lifespan (Arantes-Oliveira et al., 2002; Sutphin and Kaeberlein, 2009). We incubated the worms at 20 °C and observed them every 2–3 days. Living worms were recorded whilst dead worms were identified as having a straight body with no response to a thin platinum wire stimulus, then removed from the medium.

2.5. C. elegans reproduction assay

Age synchronized L1 larvae were moved to semi-NGM plates seeded with different diets and allowed to grow into young adults. Using a COPAS Biosort (Union Biometrica Inc., Somerville, MA, USA), one L4 nematode was added to each well of a 96-well plate, and the volume was made up to 120 µl with K-medium (51 mM NaCl, 32 mM KCl) alongside a feeding source (*E. coli* or SMB) (Boyd et al., 2010). The bacterial concentration was determined by measuring the optical density at 600 nm immediately before media was added. The nematodes were incubated for 72 hr, after which adults and offspring were aspirated using a COPAS Biosort. The total number of worms per well were recorded.

In all reproductive toxicity tests, worms were constantly exposed to Cd from the germ-free Larva 1 stage to young adult stage with developed gonads, L4. Upon reaching the young adult stage reproductive experiments were carried out according to the methods described above.

2.6. C. elegans multi-generation analysis

Multi-generational effects of SMB were investigated for 10 generations with and without exposure of Cd. Worms were exposed to Cd throughout the developmental stages. L4 *C. elegans* were randomly selected from the first day offspring of the previous generation and used to produce the next generation. In this way, reproductive tests were carried out for 10 generations (Contreras et al., 2012).

2.7. C. elegans gene expression analysis

For *C. elegans* gene expression analysis, RNA was extracted from samples using RNA extraction kits (NucleoSpin, Macherey-Nagel, Germany), then cDNA was synthesized using an oligo (dT) primer (Bio-Rad laboratories, Hercules, CA, USA). qRT-PCR was performed using a two-step RT-PCR method with IQTM SYBR Green SuperMix (Bio-Rad, Hercules, CA, USA). Four immune response genes were representatively selected, consisting of *clec-67* (C-type LECtin), *irg-2* (Infection Response protein), *lys-2* (LYSozyme), and *pmk-1* (Mitogen-activated protein kinase) (Roh et al., 2006; Troemel et al., 2006; Eom et al., 2015). Four xenobiotic and stress response genes were also selected, consisting of *cdr-2* (Cadmium Responsive), *mtl-2* (MeTaLlothionein), *sod-3* (superoxide dismutase), and *ugt-1* (UDP-GlucuronosylTransferase) (Roh et al., 2007, 2010). Gene expression was normalized using the housekeeping gene *pmp-3* and the change of expression was calculated the based on the $2^{-\Delta\Delta CT}$ calculations (Livak and Schmittgen, 2001; Hoogewijs et al., 2008). The primers were constructed based on sequences retrieved from the *C. elegans* database (www.wormbase.org). A description of the genes and their PCR primers are presented in Supplementary Table 1.

2.8. Statistical analysis

The significance of differences among and between treatments was tested statistically using two-tailed *t*-test and one-way analysis of variance (ANOVA) followed by a post-hoc test (Tukey test, $p < 0.05$). Statistical analyses were carried out using IBM SPSS 20.0 (SPSS Inc.), and graphs were prepared using SigmaPlot (Version 12.0). The dose–response relationship was estimated using R Studio software and the "drc" package (Ritz et al., 2015). A four-parameter log-logistic model was used to determine the regression. The effective concentration (EC) affecting the toxicity of the population was determined numerically from dose–response curves.

3. Results and discussion

3.1. C. elegans gut microbial community after exposure to SMB

SMB was isolated from the natural soil of a citrus farm in Jeju, Korea. Microbial community analysis was conducted on the SMB, OP50-fed and SMB-fed *C. elegans* (Fig. 1). The majority phyla of SMB were identified as Proteobacteria (90.24%) with less than 10% being Firmicutes (9.74%) (Fig. 1A). Within Proteobacteria, genera were present at the following levels: *Citrobacter* (26.49%), *Raoultella* (21.03%), *Enterobacter* (16.09%), *Providencia* (10.9%), and *Kluyvera* (4.71%). *Bacillus* (9.18%) was the most abundant Firmicutes genus (Fig. 1B). Proteobacteria (93.93%) was also a major phylum found in the *C. elegans* gut when fed with the SMB, with a small number of Actinobacteria (6.04%) (Fig. 1A). In the Proteobacteria, *Stenotrophomonas* (59.21%) and *Raoultella* (24.67%) were the major genera (Fig. 1B). *Stenotrophomonas* accounted for less than 1% of the bacteria in SMB, however in SMB-fed *C. elegans* it was more prevalent than *Raoultella*. Within the Actinobacteria, *Rhodococcus* (4.98%) accounted the majority of the phylum. The major difference between SMB and SMB- fed *C. elegans* was the increased proportion of the Actinobacteria phylum. The diversity of the microbial community was determined using various diversity index as presented in Table 1. Species richness in the bacterial community of SMB and SMB-fed *C. elegans* was compared using the abundance-based coverage estimator (ACE), Chao1 richness, JackKnife, Shannon diversity, and Good's coverage indices. All diversity index values were higher in the SMB than in SMB-fed *C. elegans* in two to three times. The number of operational taxonomic units per number of sequence reads in pyrosequencing was presented as rarefaction curve (Supplementary Fig. 1). This curve also showed higher taxonomic unit in SMB than in SMB-*fed C. elegans*. Overall results suggested a loss of SMB diversity after colonization of the *C. elegans* gut. Different living environment of SMB between culture in LB media and *C. elegans* gut would affect bacterial diversity of SMB.

3.2. Effect of SMB on C. elegans life history traits

The effect of the SMB on *C. elegans* life history traits were evaluated by comparing the response of worms fed with OP50, using survival and reproduction as endpoints (Fig. 2). SMB-fed worms had longer lifespans by approximately one week (Fig. 2A) and also showed increased reproductive ability. To investigate whether this increased fitness due to SMB feeding was sustained over generations, multi-generation tests were performed for 10 generations (Fig. 2B). Higher fertility was observed in worms fed with SMB than OP50 for all 10 generations tested.

We hypothesized that SMB feeding altered the exo/endobacterial environment of the worm, which may lead to changes in transcriptomic/metabolomic activity and ultimately to changes in phenotype (Coolon et al., 2009). Many studies have demonstrated the relationship between the bacterial diet and physiological characteristics of *C. elegans*. Bifidobacteria, known as the representative probiotics of humans, have been reported to play a beneficial role in *C. elegans* (Ikeda et al., 2007; Komura et al., 2013). In a study by Ikeda et al. (2007), Lactobacilli and Bifidobacteria cell wall components were suggested to play a responsible role for immunostimulation. In our study, though the proportion of Bifidobacteria present in the SMB was not significantly high, presence of these bacteria might contribute to stimulate immune response. The proportion of the *Rhodococcus* genus increased by approximately four times in SMB-fed worms compared to OP50-fed worms (Fig. 1B). Interestingly, *Rhodococcus erythropolis* ketosteroid hydroxylase is homologous to the DAF-36 of *C. elegans* (Rottiers et al., 2006). DAF-36 is homologous to the catalytic subunit of Rieske-like ring hydroxylating oxygenase and the DAF-12 ligand is produced during the process of daf-36 Rieske-like oxygenase participation due to its molecular identity. The DAF-12 hormone is known to regulate *C. elegans* dauer diapause, gonadal outgrowth, and adult longevity in the germline pathway. The homology of DAF-36 to kshA, a subunit of ketosteroid hydroxylase in *Rhodococcus erythropolis* may have functions in sterol metabolism, which can significantly affect lifespan (Wollam et al., 2011). Apolipoprotein B (ApoB) is a protein secreted by enterocytes and hepatocytes, which plays an important role in the transport of cholesterol inside and outside the cell (Matyash et al., 2001). It has also been shown that ApoB can be upregulated by intestinal microbial communities (Shim et al., 2002). Cholesterol is crucial to nematodes as it promotes the growth and development of embryos in oocytes and acts as a precursor to hormones which stimulate the development of larvae into adults (Hieb and Rothstein, 1968). *C. elegans* is auxotrophic for sterols and does not possess a sterol biosynthesis enzyme, therefore, cholesterol must be externally supplied. It can be inferred that a gut microbial community changed by diet can modify the host's energy utilization, reconstruct signaling networks, and regulate metabolite trafficking to improve the life expectancy of the host (Kenyon, 2010; Samuel et al., 2016).

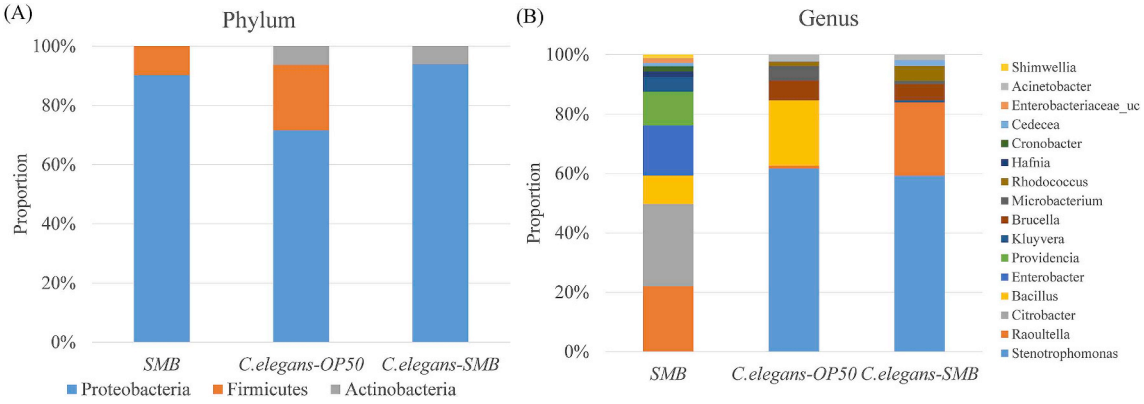

Fig. 1. Bacterial communities in SMB, OP50-fed, and SMB-fed *C. elegans* (A) Phylum (B) Genus. The nomenclature of phylotypes is based on the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net). *C. elegans* was fed with OP50 or SMB from L1 larva to young adult.

Table 1
Bacterial diversity indices of SMB and SMB-fed *C. elegans*.

Target reads	Valid reads	OTUs[a]	Ace	Chao1	JackKnife	Shannon	Goods Lib. Coverage
SMB	99529	166	176.02	168.78	184	2.53	99.98
SMB-fed *C. elegans*	26239	51	57.71	52.47	59	1.26	99.97

[a] The operational taxonomic units (OTUs) were defined by 3% dissimilarity.

3.3. Effect of SMB on toxic response to cadmium exposure in C. elegans

After identifying the beneficial effects of the SMB in *C. elegans*, we then studied its role in the chemical toxicity response, using Cd exposure. The toxicity of Cd was compared between OP50- and SMB-fed worms using reproduction as an endpoint (Fig. 3A). A clear dose-response relationship was found in both groups. SMB-fed worms showed a reduced toxic response compared to OP50-fed worms at all concentrations tested in a statistically significant way. Based on the results, EC_{10}, EC_{20}, EC_{50}, and EC_{90} values were estimated, which showed that the toxicity of Cd in SMB-fed worms decreased by approximately 50% compared to that of OP50-fed worms (Table 2).

To find further whether Cd toxicity rescued by SMB was maintained over generations, multi-generation toxicity tests were conducted. Based on the EC_{20} values of Cd, which were 0.92 and 1.79 mg/L in OP50 and SMB fed worms, respectively, worms were exposed to 1 mg/L of Cd for 10 consecutive generations and reproduction was compared (Fig. 3B). SMB-fed worms showed more tolerant behavior than OP50-fed worms across the generations. Beneficial effect of SMB on *C. elegans* fitness, as shown in Fig. 2, seems to confer *C. elegans* to have better stress response mechanism to chemical insult, such as, Cd exposure.

In this study, SMB supported host-beneficial effect in fitness and stress response, whereas, in our previous study we found host-detrimental effect of microbe in chemical stress response (Kim et al., 2018). In that study, the toxic response of *C. elegans* to Cd exposure was exacerbated by pre-treating with pathogenic microbes, *Pseudomonas aeruginosa* strain PA01, compared to untreated worms. In the same study with silver nanoparticles, we found that the acquired tolerance of *C. elegans* was due to boosted immunity resulting from PA01 infection. This was shown by the immune response genes activated by pathogen infection, which remained activate after exposure to silver nanoparticles.

We also postulated that the acquired tolerance of SMB-fed worms to Cd exposure might play a role in better defense against toxicity. To elucidate this, gene expression analysis was performed using a panel of stress response (i.e. *mtl-2, cyp35a2, cdr-2, ugt-1, and sod*-3) and immune response genes (i.e. *pmk-1, lys-1, clec-67, irg-2, and lys-2*). They are representative genes in *C. elegans* stress and immune response (Troemel et al., 2006; Cristina et al., 2009; Zhang et al., 2011; Miriyala et al., 2012; Keshet et al., 2017), and detailed description of those genes is in Supplementary Table 1. Expression of those genes in SMB-fed worms in response to OP50-fed worms was examined (Fig. 4). Increased expression was observed on all genes tested, except for, *mtl-2, cyp35a2* and *pmk-1*, suggesting SMB feeding has a potential to stimulate stress and immune response in *C. elegans*. All immune response genes were up-regulated in the SMB group compared with the OP50 group except *pmk-*

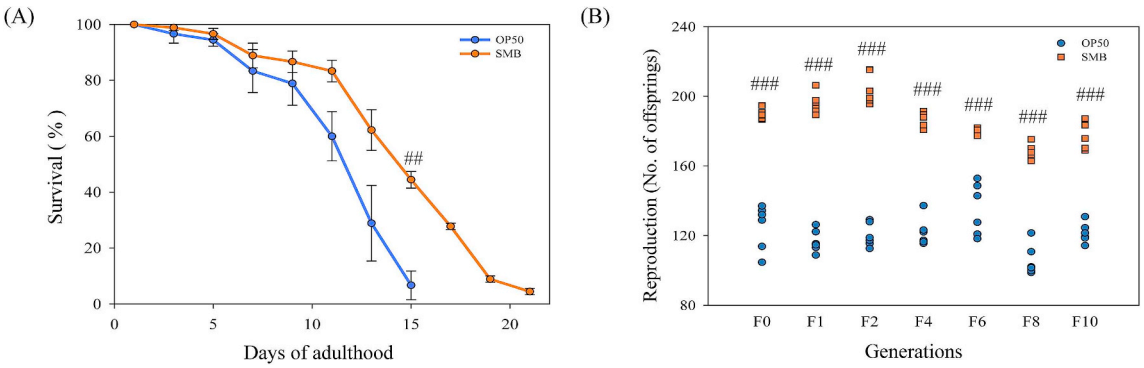

Fig. 2. Effect of soil microbiome on *C. elegans* life history traits (A) Survival (B) Reproduction. The results are showed as mean values compared by two-tailed *t*-test (n = 90, mean ± SEM, p value: #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$).

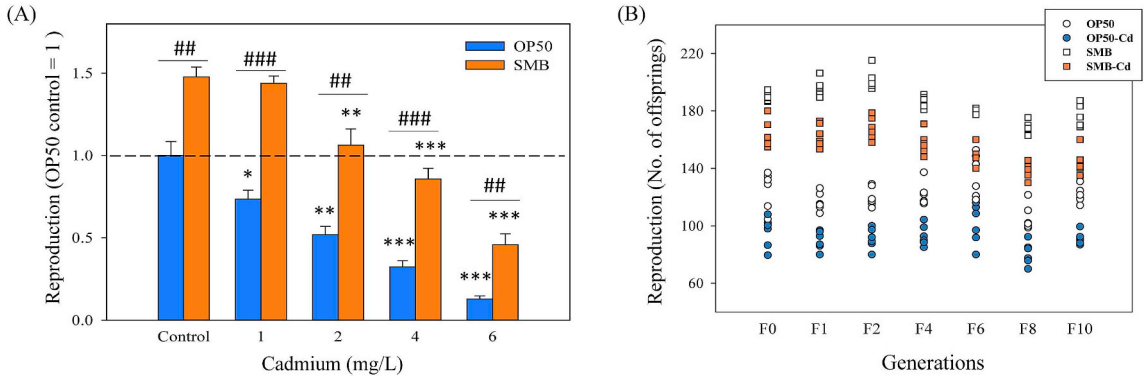

Fig. 3. Effect of Cd on reproduction of *C. elegans* fed with OP50 and SMB (A) Reproduction-Single generation with different exposure concentration (B) Reproduction-multigeneration. The results are showed as mean values compared by one-way ANOVA. The statistical difference between control and Cd exposed groups (*, $p < 0.05$; **, $p < 0.01$) and between OP50 fed and SMB fed groups (#, $p < 0.05$, ##, $p < 0.01$, and ###, $p < 0.001$). (control = 1, n = 24, mean ± SEM).

Table 2
EC values estimated in Cd exposed OP50- and SMB-fed *C. elegans* using 72 hr reproduction as endpoint.

Group	EC (95% Confidence interval) (mg/L)	
OP50-fed *C. elegans*	EC_{10}	0.55 (0.37–0.73)
	EC_{20}	0.92 (0.70–1.14)
	EC_{50}	2.23 (1.96–2.51)
	EC_{90}	9.10 (6.49–11.71)
SMB-fed *C. elegans*	EC_{10}	1.03 (0.69–1.36)
	EC_{20}	1.79 (1.39–2.20)
	EC_{50}	4.67 (3.99–5.34)
	EC_{90}	21.23 (13.07–29.38)

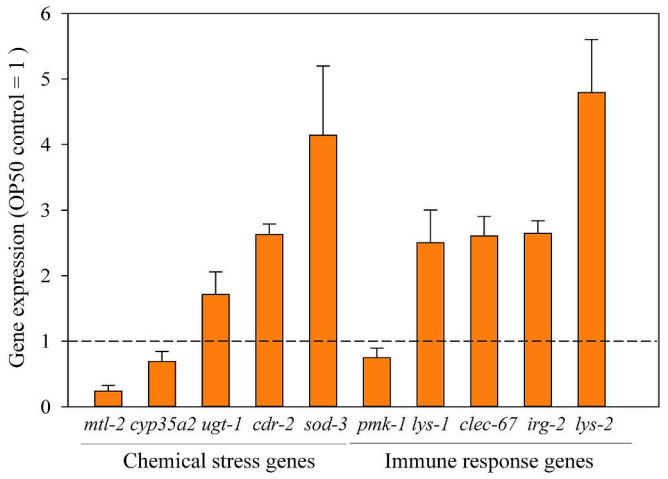

Fig. 4. Expression of stress and immune response genes in SMB-fed *C. elegans*. The results were showed as relative values compared to OP50-fed *C. elegans* housekeeping gene (control = 1, n = 3, mean ± SEM) *pmp-3* was used as housekeeping gene.

1. We found that the SMB feeding caused to upregulate the stress- and immune-related genes of *C. elegans*, meaning that the host-gut microbiota interaction may lead to transcriptional activation contributing to a beneficial health outcome.

We then selected 3 genes at each category (i.e. *mtl-2, cdr-2, ugt-1* for stress response genes, *pmk-1, clec-67, lys-2* for immune response genes) to see how their expression altered after Cd exposure (Fig. 5). In SMB-fed *C. elegans*, increased expression by Cd exposure was significant in all genes, except for *mtl-2* (Fig. 5). Taken that selected genes were mostly involved in oxidative stress defense or immune response in *C. elegans*, as described in Supplementary Table 1, boosted stress response mechanism by SMB obviously plays important role in defense to Cd exposure. Overlap and interplay of the immune and the stress response systems in *C. elegans* (Keshet et al., 2017) seem to hold also true in this study with Cd exposure and SMB feeding. The expression pattern of *mtl-2* was completely different than other genes. MTL-2 is a well-known biomarker of metal exposure, including Cd (Roh et al., 2009b). Dramatic increase of *mtl-2* gene by Cd exposure in OP50-fed worms but not in SMB worms may suggest detoxification of Cd may take place in SMB-fed worms. Further, more sophisticated study is needed on the potential role of SMB to chemical insult, which in turn, would affect host defense mechanism.

3.4. Effect of cadmium on C. elegans gut microbial community

Based on the organism- and gene expression level toxicity results (Figs. 3–5), we assumed that gut microbial communities modified by SMB would have beneficial effects on the host. We therefore examined the gut microbial community caused by Cd exposure in OP50- and SMB-fed worms (Fig. 6). We first investigated whether Cd exposure could cause changes in the SMB itself. Cd exposure did not lead to any significant changes in microbial community of the SMB itself. We then investigated effect of Cd on the *C. elegans* gut microbiome in OP50- and SMB-fed worms. In OP50-fed worms, before Cd exposure Proteobacteria (71.56%) was the most dominant phylum followed by the Firmicutes (22.07%) and Actinobacteria (6.35%). *Stenotrophomonas* (61.39%) was the dominant genus in the Proteobacteria phylum, *Bacillus* genus (21.95%) in the Firmicutes and *Microbacterium* (4.87%) genus in Actinobacteria. Cd exposure caused dramatic changes to the microbial community in OP50 fed worms (Fig. 6). It was suggested that Firmicutes, which are relatively resistant to Cd, were better adapted to the intestinal environment after exposure to Cd, resulting in proliferation. Hence Cd exposure caused severe changes in microbial community composition in OP50-fed worms' gut. In SMB fed worms, before the chemical exposure Proteobacteria (93.93%) was the most prevalent phylum. As with OP50-fed worms, *Stenotrophomonas* (59.21%) and *Rhodococcus* (4.98%) were the most abundant genera in the Proteobacteria and Actinobacteria, respectively. Cd exposure resulted in an increase in Actinobacteria (13.43%). The proportion of the *Brucella* genus (11.53%) in Proteobacteria and *Rhodococcus* (12.31%) in Actinobacteria increased by about 2 times following Cd exposure (Fig. 6). These genera can also be assumed to be resistant to Cd due to proliferation (Lorenz et al., 2006). Differently from OP50-fed worms, in SMB group, even after exposure to Cd, the dominant genus and phylum remained the same as before exposure. The gut microbial community of the SMB-fed worms was not significantly affected by Cd exposure. It seemed gut microbial community acts as buffer zone toward external stress such as, Cd exposure.

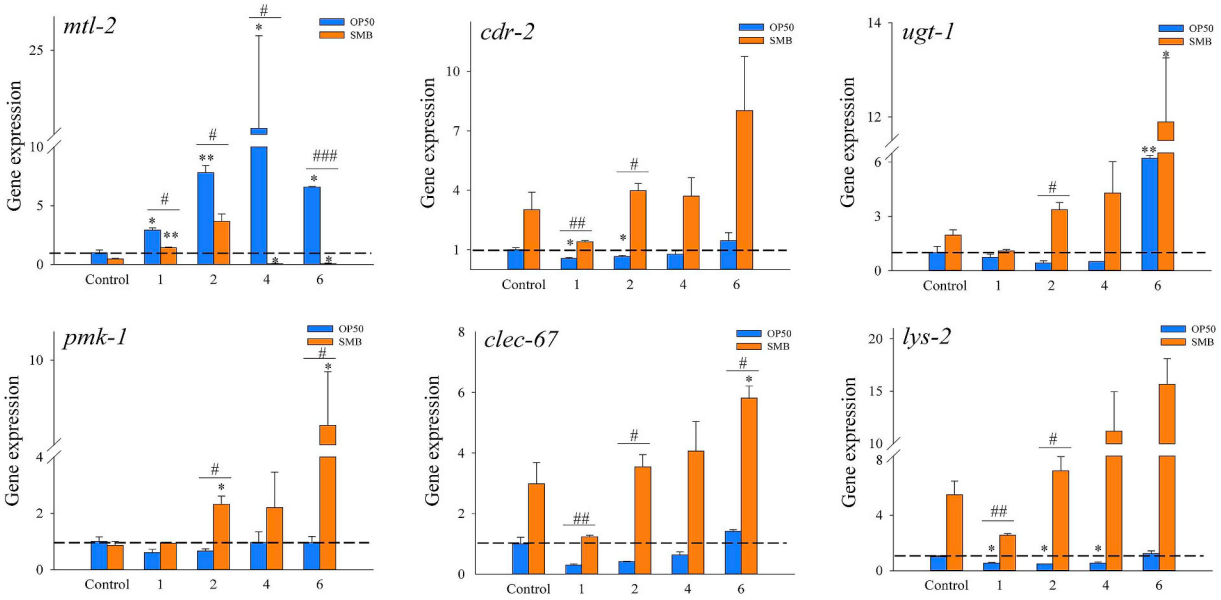

Fig. 5. Effect of Cd exposure on the expression of stress and immune response genes in *C. elegans* fed with OP50 or SMB. The results were showed as mean values compared to OP50-fed group control (control = 1, n = 3, mean ± SEM). The statistical difference between control and Cd exposed groups (*, $p < 0.05$; **, $p < 0.01$) and between OP50 fed and SMB fed groups (#, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$) were analyzed by one-way ANOVA. *pmp-3* was used as housekeeping gene.

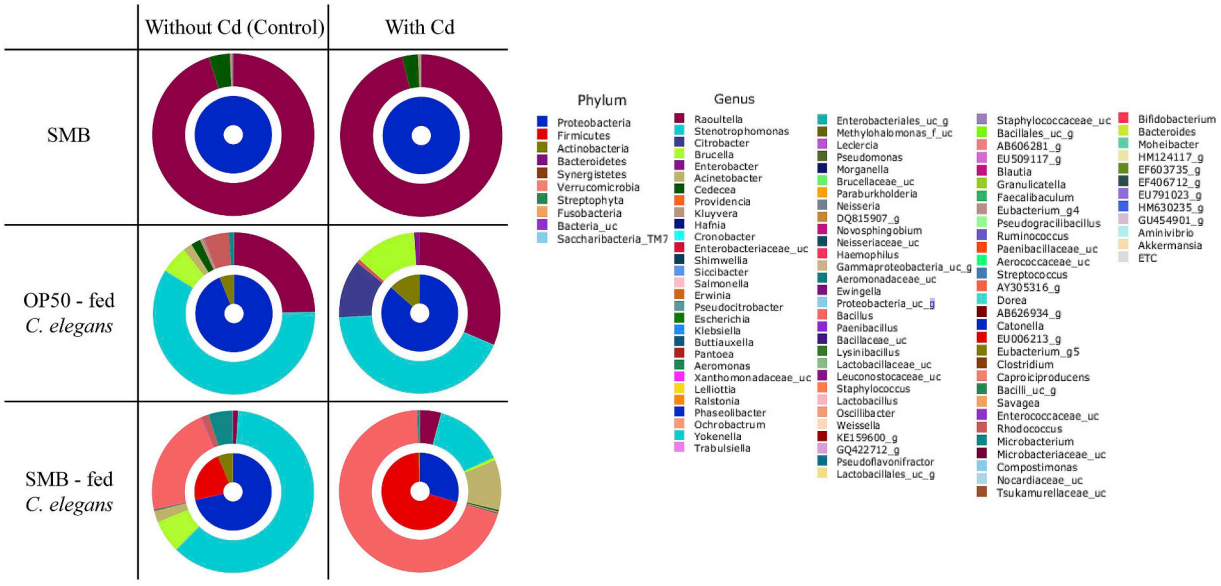

Fig. 6. Effect of Cd on microbial communities in SMB, *C. elegans* fed with OP50 and *C. elegans* fed with SMB, with/without Cd treatment. The inner and outer pies represent the composition of phyla and genera, respectively. The nomenclature of phylotypes is based on the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net/).

4. Conclusion

In this study, effect of gut microbial community on the toxicity response to Cd was investigated in *C. elegans* comparing the response between OP50- and SMB-fed worms. Overall results suggest that SMB-fed worms showed more resistant to Cd by activation of chemical stress and immune response gene expression and also by maintaining gut microbial communities stable. Our results highlight the importance of the gut microbiota in host stress response.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109777.

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