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Lysinibacillus sphaericus mediates stress responses and attenuates arsenic toxicity in *Caenorhabditis elegans*

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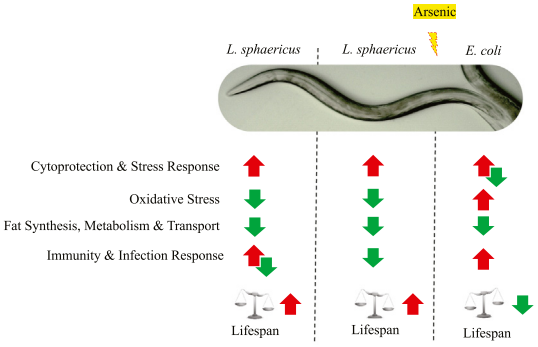
HIGHLIGHTS

- *L. sphaericus* diet extends *C. elegans* lifespan compared to *E. coli* diet.
- *L. sphaericus* reduced arsenic-mediated stress response and ROS in *C. elegans*.
- *L. sphaericus* increased *C. elegans* immunity, fat metabolism and resilience to arsenic.
- *E. coli* fed *C. elegans* displayed fat accumulation, increased ROS and shorter lifespan.

GRAPHICAL ABSTRACT



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ABSTRACT

Exposure to toxic metals alters host response and that leads to disease development. Studies have revealed the effects of metals on microbial physiology, however, the role of metal resistant bacteria on host response to metals is unclear. The hypothesis that xenobiotic interactions between gut microbes and arsenic influence the host physiology and toxicity was assessed in a *Caenorhabditis elegans* model. The arsenic-resistant *Lysinibacillus sphaericus* B1CDA was fed to *C. elegans* to determine the host responses to arsenic in comparison to *Escherichia coli* OP50 food. *L. sphaericus* diet extended *C. elegans* lifespan compared to *E. coli* diet, with an increased expression of genes involved in lifespan, stress response and immunity (*hif-1, hsp-16.2, mtl-2, abf-2, clec-60*), as well as reduced fat accumulation. Arsenic-exposed worms fed *L. sphaericus* also had a longer lifespan than those fed *E. coli* and had an increased expression of genes involved in cytoprotection, stress resistance (*mtl-1, mtl-2*) and oxidative stress response (*cyp-35A2, isp-1, ctl-2, sod*-1), together with a decreased accumulation of reactive oxygen species (ROS). In comparison with *E. coli*, *L. sphaericus* B1CDA diet increased *C. elegans* fitness while detoxifying arsenic induced ROS and extending lifespan.

1. Introduction

Heavy metal pollutants in the environment pose a serious ecological risk to living organisms. The first biota that undergoes direct and indirect negative impact of heavy metals are the microorganisms. Metals not only affect microorganisms by reducing their number, diversity, biochemical activity, but also leads to the establishment of metal tolerant microbial populations (Babich and Stotzky, 1985; Ben Fekih et al., 2018; Giller et al., 1998; Prabhakaran et al., 2016). Arsenic is one of the most widely distributed metalloid, commonly found in soil and water, and exists in either organic or inorganic forms. The degree of toxicity of arsenic species depends on its oxidation state, where the +5 oxidation state (arsenate) is presumed to be less toxic than the +3 oxidation state (arsenite). Both inorganic and organic species bioaccumulate in animals and plants, and pose a health concern worldwide (Bhattacharjee et al., 2013; Naujokas et al., 2013; Tchounwou et al., 2019).

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Chronic exposure to arsenic through contaminated food and water is a global concern (Cubadda et al., 2017; Gundert-Remy et al., 2015). Arsenic exposure in humans, is known to cause cancer, diabetes, and cardiovascular disorders (Choiniere and Wang, 2016; Naujokas et al., 2013; Renu et al., 2018; Tchounwou et al., 2019). Research conducted on arsenic exposed human populations, as well as vertebrate and invertebrate animal models proposed several mechanisms of arsenic toxicity, such as aberrant cellular signaling (Druwe and Vaillancourt, 2010), immunotoxicity (Bellamri et al., 2018), abnormal lipid metabolism (Renu et al., 2018), and epigenetic alterations (Argos, 2015). At the level of host gut microbiota, arsenic exposure is also shown to alter the microbial diversity, which affects the accumulation and bioavailability of arsenic species, and perturbs the host and microbial physiological responses (Chi et al., 2017; Dong et al., 2017; Gokulan et al., 2018; Lu et al., 2014; Richardson et al., 2018).

Variability in response to arsenic toxicity is observed in humans and animal models, and points at adaptative arsenic detoxification mechanisms. These mechanisms have developed through genetic modifications (Schlebusch et al., 2015), nutritional factors (Gamble et al., 2005), and xenobiotic interactions with the gut microbial communities (Coryell et al., 2018). Recent evidence also suggests a role for gut colonizing microbes in the conversion of arsenic species both *in vitro* and *in vivo* (Pinyayev et al., 2011; Van de Wiele et al., 2010), which can affect the host.

Bacterial adaptation and resistance to arsenic stress has been extensively studied and is dependent upon several mechanisms (Yan et al., 2019). Presence of arsenate resistance (*ars*) and arsenate oxidation (*aio / arx*) genes gives bacteria the ability to convert toxic arsenite to lesser toxic arsenate species, while arsenite reduction (*arr*AB) system converts the arsenate back to arsenite within the periplasm. Alternatively, arsenic methylation genes assist bacteria in converting toxic inorganic arsenic species to the less toxic methylated arsenics species thus contributing to detoxification (Yan et al., 2019). *Lysinibacillus sphaericus* B1CDA is an arsenic tolerant and remediating bacterium and was shown to tolerate up to 500 mM arsenics. *L. sphaericus* B1CDA possesses several genes known to provide resistance to arsenic and metal detoxification (Rahman et al., 2016; Rahman et al., 2014). Most studies have reported effects of metal contamination on the physiology of metal tolerant microbes, however the subsequent impact of these microbes on the host is understudied.

Caenorhabditis elegans is a bacterivorous nematode that thrives in soil microenvironments rich with microorganisms. It feeds on bacteria and fungi, and is thus naturally colonized with a mixture of microorganisms from their environment (Schulenburg and Félix, 2017). In the laboratory, nematodes are usually monoxenically grown with the auxotrophic strain *Escherichia coli* OP50 (Stiernagle, 2006). The success of *C. elegans* as an important model organism has been ascribed to its short life cycle, ease of maintenance, completely characterized genome, availability of tools for genetic manipulation, high degree of homology to human genome, and similar metabolic pathways to humans (Kaletta and Hengartner, 2006), making it an interesting model to study host-microbiota interactions (Zhang et al., 2017).

Based on the study hypothesis that xenobiotic interactions between gut microbes and arsenic influence the host physiology and thus toxicity, *C. elegans* physiology was investigated in nematodes fed the arsenic tolerant and accumulating bacteria, *L. sphaericus* B1CDA, in the presence and absence of arsenic. Lifespan, fat accumulation, reactive oxygen species (ROS) accumulation and gene expression were used to assess the physiological responses of the nematode.

2. Materials and methods

2.1. Metal solutions

Sodium dioxoarsenate ($NaAsO_2$) and di-Sodium hydrogen arsenate heptahydrate (Na_2AsO_4 7.H_2O) were purchased from Sigma and Merck, respectively. A 1.0 g/L metal stock solution of each metalloid was prepared in milli-Q water.

2.2. Bacterial strains and growth conditions

Lysinibacillus sphaericus B1CDA is a Gram-positive, rod-shaped and motile bacterium. At the genetic level, *L. sphaericus* B1CDA possesses arsenic biotransformation *(acr3, arsR, arsB and arsC)* and metal resistance genes (Rahman et al., 2016; Rahman et al., 2014). *L. sphaericus* B1CDA and *Escherichia coli* OP50 were maintained in Luria Bertani (LB) agar/broth (BD Difco). For *C. elegans* experiments with metals, the bacteria were grown overnight in LB broth at 37 °C and 180 rpm to an optical density (λ = 600 nm) of 0.9 and subsequently washed and re-suspended in equivalent volume of M9 buffer (22 mM KH_2PO_4, 42.2 mM Na_2HPO_4, 85.5 mM NaCl, 1 mM $MgSO_4$). The bacterial suspensions in M9 buffer were incubated in 10 mg/L arsenite ($NaAsO_2$) or arsenate (Na_2AsO_4 7H_2O) solution, or control K-media (51 mM NaCl, 32 mM KCl) for 1 h at 37 °C and shaking at 180 rpm. Finally, the bacterial suspensions were centrifuged at 2000 ×*g* for 5 min, and the bacterial pellets were resuspended in 300 μL of K-media to 20× the original bacterial density. The bacterial suspensions were used to prepare bacterial lawns on nematode growth medium (NGM) plates for *C. elegans* exposures.

2.3. Synchronizing of C. elegans

Bristol wild type N2 *C. elegans* strain was maintained at 20 °C on nematode growth medium (NGM) with *E. coli* OP50 as food according to previously established protocols (Stiernagle, 2006). To obtain age-synchronized worms, adult hermaphrodites were washed and collected in M9 buffer and treated with alkaline sodium hypochlorite solution (20% NaClO, 3% NaOH). The released eggs were washed three times, collected in M9 buffer, and allowed to hatch overnight without food at constant rotation. Age synchronized L1 larvae were used for subsequent experiments.

2.4. C. elegans exposures to Arsenic

For the analysis of metal toxicity on *C. elegans*, a concentration of 10 mg/L arsenite or arsenate was chosen based on the lowest observed adverse effect concentration (LOAEC) (Liao and Yu, 2005; Yu et al., 2016). For *C. elegans* exposures to the metals, NGM plates were prepared with final metal concentrations of 10 mg/L in K-media and were supplemented with bacteria pre-treated for 1 h with 10 mg/L arsenite or arsenate in K-media.

2.5. C. elegans lifespan

Age synchronized L1 worms were transferred onto NGM prepared with arsenite or arsenate to a final concentration 10 mg/L or K-media, supplemented with 50 μL of bacterial food (*E. coli* OP50 or *L. sphaericus* B1CDA) in a 60 mm petri dish (Sarstedt). Upon reaching L4 stage, approximately 50 worms (10 worms/replicate/condition) were transferred to their respective NGM media supplemented with 5-fluoro-2-deoxyuridine (FUdR) as previously described and live nematodes were counted daily (Lionaki and Tavernarakis, 2013). Worms were considered alive based on their movement and pharyngeal pumping, whereas worms were considered dead when they did not respond to tapping or poking with a platinum wire. *C. elegans* life span experiments were repeated twice with at least 5 replicates per condition per experiment.

2.6. RNA extraction and qRT-PCR

Approximately 1500 age synchronized L1 worms were dropped onto NGM prepared with K-media or with the different metals (4 replicates / exposure) supplemented with bacterial food and harvested upon reaching L4. The exposed worms were collected in 1.5 mL microfuge tubes and washed with M9 buffer. For RNA extraction, the worms were transferred to 2 mL cryotubes containing 1.4 mm ceramic beads (Precellys), with 700 μL of Tri Reagent (Sigma-Aldrich) and frozen overnight at −80 °C. Worms were thawed and lysed at 6000 rpm thrice for 20 s with a 20 s pause between each cycle in a homogenizer (Precellys), and RNA was isolated

using the Directzol RNA kit (Zymo Research) following manufacturer's protocol. The RNA was quantified using the Nanodrop spectrophotometer (DeNovix) and cDNA was synthesized using 500 ng of RNA with the qScript cDNA synthesis kit (Quanta Biosciences). qRT-PCR of 46 genes (Supplementary Table 1) was performed using KAPA SYBR FAST qPCR Kit (Kapa Biosciences). Thermocycling conditions for qRT-PCR consisted of a denaturation step for 3 min at 95 °C followed by 35 cycles of 95 °C for 2 s and 60 °C for 30 s in CFX 96 Real time PCR detection system (Biorad). The values were normalized using the reference gene *iscu-1* (Y4510D.4) as described earlier (Kumar et al., 2015; Rai et al., 2019). The relative fold expression was normalized to the *C. elegans* fed with *E. coli* OP50 in K-media control, and the fold expression was calculated using the ΔΔCt method. Gene expression experiments were done at least twice with four technical replicates per condition.

2.7. Reactive oxygen species assay

Intracellular accumulation of ROS was performed as described previously (Thabit et al., 2019) with some modifications. Synchronized L1 nematodes (1000 worms per exposure) grown on NGM plates with K-media, with different metals, or 70 μM paraquat (positive control, Acros organics) were harvested upon reaching L4 / young adults (44 h) stage and washed in M9 buffer to remove excess bacteria. The worms were then incubated in 1 mL of M9 buffer containing 50 μM of 5′,6′-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA) (Invitrogen) for 1 h at room temperature in the dark. Subsequently worms were washed in M9 buffer to remove excess dye, paralyzed on cover slips using 25 mM Levamisole (Sigma), and imaged using a Scanning Confocal microscope (Fluoview 1000, Olympus IX81) with the FITC channel (465–495 nm excitation/515–555 nm emission). The fluorescence intensity of individual worms was measured using NIH Image J 1.46r software.

2.8. Fat staining

Nile red (NR) and oil red O (ORO) staining was performed as previously described (Rai et al., 2019). L1 exposed nematodes (1000 worms/exposure) were grown on *E. coli* or *L. sphaericus* bacterial lawns on nematode growth plates supplemented with metals or K-media. Worms were harvested upon reaching L4/ young adults (44 h) stage and washed in phosphate buffered saline containing 0.01% Triton X-100 (PBS-T), fixed in 40% isopropanol, and stained with Nile red or oil red O (Sigma). The nile red stained worms were imaged using FITC channel (465–495 nm excitation/515–555 nm emission) in Fluoview 1000 Scanning Confocal microscope (Olympus) and the oil red O stained worms were visualized using bright field illumination microscope (Olympus BX51). The staining intensity of individual worms was measured using NIH Image J 1.46r software. For oil red O stained worms, the background light intensity was corrected using the background correction function, and micrographs were inverted before quantification in ImageJ software.

2.9. Statistical analysis

Statistical significance of qRT-PCR, Nile red, oil red O staining, body area, and ROS assay were determined using either unpaired *t*-test, or the one-way analysis of variance followed by Dunnett posttest or Tukeys posttest for multiple group comparison, and the differences were considered significant if the *p*-values were < 0.05 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). For the lifespan analysis, Log rank (Mantel-Cox) test was used to determine statistical significance. All the statistical analyses were performed using GraphPad Prism 8 (GraphPad software). Principal component analysis was performed to explain the variation within the models using SIMCA software version 13.0.3 (Umetrics) at a significance level of 0.05. Acceptable models were developed to explain the variation within the data which fulfilled both the goodness of fit (R2X > 0.7) and goodness of prediction (Q2X > 0.4) metrics.

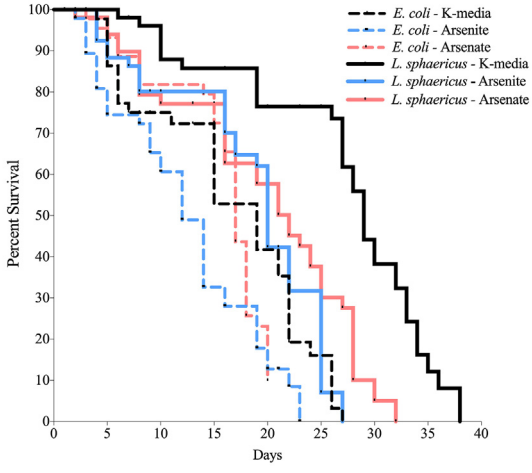
3. Results

3.1. L. sphaericus B1CDA fed C. elegans presented extended lifespan when compared to E. coli in the presence of arsenics

C. elegans fed *L. sphaericus* had increased body size, brood and life span in previous reports (Go et al., 2014). Since the *L. sphaericus* B1CDA strain is resistant to arsenics and can transform between arsenite and arsenate, we postulated that it would confer beneficial effects on *C. elegans* lifespan during arsenic exposure when compared to the standard *E. coli* OP50 fed worms. Worms fed *E. coli* OP50 had a median survival of 19 days (Fig. 1), whereas worms fed *L. sphaericus* B1CDA had a median survival of 29 days, thus *L. sphaericus* B1CDA diet resulted in a significant increase in the median lifespan of the worms (+10 days, $p < 0.001$) (Fig. 1B). The effect of the two arsenic species on *C. elegans* lifespan was determined by feeding *C. elegans* with arsenic pre-treated *E. coli* or *L. sphaericus* on solid NGM plates supplemented with 10 mg/L arsenite or arsenate. The median survival of worms fed arsenite pre-treated *E. coli* OP50 was significantly reduced by 37% ($p = 0.003$), whereas arsenate only reduced survival by 11% ($p = 0.098$) (Fig. 1). Although the median survival of *C. elegans* fed arsenite and arsenate pre-treated L. *sphaericus* was reduced by a greater number of days, the lifespan decreased by 31% ($p < 0.001$) and 24% ($p < 0.001$), respectively, when compared to the K-media control (Fig. 1).

Despite a clear lifespan reduction in *C. elegans* fed with arsenite pre-treated bacteria, the worms had a significant increase in median survival by 8 days ($p < 0.001$) when fed with L. *sphaericus* compared to the arsenite pre-treated *E. coli* fed worms and this was comparable to the median survival in *E. coli* control (K-media) fed worms (Fig. 1). In contrast, arsenate

A.



B.

Comparision within groups	*E. coli*			*L. sphaericus*		
	K-media	Arsenite	Arsenate	K-media	Arsenite	Arsenate
N	47	47	45	54	54	57
Median survival (days)	19	12	17	29	20	22
Median survival change (days)	----	- 7	- 2	----	- 9	- 7
p-value	----	0.003	0.098	----	<0.001	<0.001
Significance	----	**	ns	----	***	***
Comparision across groups	K-media ⟷ K-media Arsenite ⟷ Arsenite Arsenate ⟷ Arsenate					
Median survival change (days)				+ 10	+ 8	+ 5
p-value				<0.001	<0.001	<0.001
Significance				***	***	***

Fig. 1. Arsenite and arsenate influence lifespan of *C. elegans* fed either *E. coli or L. sphaericus*. (A) *C. elegans* fed with *E. coli* OP50 or *Lysinibacillus sphaericus* B1CDA on nematode growth media with K-media control, arsenite (10 mg/L) or arsenate (10 mg/L). (B) Summary of median survival and statistical analysis. The "-" and "+" indicates decrease and increase in lifespan, respectively. Median survival and significance of survival data were generated using Kaplan-Meier curves and Mantel-Cox test, respectively.

pre-treated *L. sphaericus* fed worms lived longer and had an increased median survival of 5 days ($p < 0.001$) compared to its *E. coli* fed counterparts (Fig. 1). This indicates that *L. sphaericus* confers a physiological benefit to the *C. elegans* leading to an increased lifespan even in the presence of arsenics, which was not seen with the *E. coli* diet.

3.2. L. sphaericus B1CDA diet altered arsenic-induced stress response gene expression in C. elegans

L. sphaericus B1CDA appears to be beneficial to *C. elegans*, however the mechanism for this effect is yet unknown. Newly hatched worms fed either *E. coli* or *L. sphaericus* were exposed to arsenate or arsenite on NGM until they reached young adults (L4/ 44 h exposure) and the expression of 15 representative genes associated with heat shock, heavy metal and oxidative stress response were examined using qRT-PCR (Supplementary Table S1). The genes were selected based on their role in metal toxicity and nematode lifespan (Murphy et al., 2003a; Uno and Nishida, 2016). Of the 15 genes tested, 5 genes were significantly modulated in response to *L. sphaericus,* while 10 genes showed no change in expression (Supplementary Table S2). Amongst the significantly modulated genes, 5 genes had increased expression in *L. sphaericus* fed worms, including *hsp-16.2* ($p = 0.007$), *mtl-1* ($p = 0.004$), *mtl-2* ($p = 0.027$), *ctl-2* ($p = 0.005$) and *cyp-35A2* ($p < 0.001$) when compared to *E. coli* fed worms. This revealed that *L. sphaericus* diet influenced the expression of genes involved in stress response.

An initial comparison was made of the gene response in *C. elegans* fed *E. coli* OP50 to arsenate and arsenite. Of the 15 genes analyzed, the expression of 6 genes was increased in arsenite exposed worms fed with *E. coli* OP50, while 9 genes showed no significant change (Supplementary Table S2). Upregulated genes included those associated with heat shock, *hsp-16.1* ($p = 0.013$), *hsp-16.2* ($p = 0.015$) and *hsp-48* ($p = 0.039$); metal response, *mtl-1* ($p = 0.002$) and *numr-1* ($p = 0.001$); and oxidative stress response *gst-4* ($p < 0.001$) and *prdx-2* ($p = 0.033$) (Fig. 2). Similarly, arsenate exposed worms fed *E. coli* OP50 showed a significant change in the expression of 8 genes (Fig. 2 and Supplementary Table S2). The gene expression increased for *hsp-16.1* ($p = 0.015$), *hsp-16.2* ($p = 0.019$), *numr-1* ($p = 0.002$) and *gst-4* ($p < 0.001$), whereas the expression of *mtl-2* ($p = 0.001$), *aip-1* ($p = 0.035$) and *prdx-2* ($p = 0.032$) decreased.

C. elegans fed *L. sphaericus* B1CDA in the control K-media were compared to those fed *L. sphaericus* B1CDA in the presence of the arsenic species. Overall, the worms exhibited a significant change in gene expression in 4 of the 15 genes analyzed (Fig. 2 and supplementary Table 2). In worms fed arsenite or arsenate pre-treated *L. sphaericus*, one gene belonging to oxidative stress- *gst-4* ($p < 0.001$) was significantly increased (Fig. 2A). While worms fed arsenite pre-treated *L. sphaericus* showed a significant decrease in expression of *cyp-35A2* ($p < 0.001$), worms fed arsenate pre-treated *L. sphaericus* presented a decrease in expression for metal response genes (*aip-1,* $p = 0.03$) and *mtl-2* ($p = 0.04$) (Fig. 2A).

In comparison to the *E. coli* fed worms in the respective arsenic species, *L. sphaericus* fed worms in arsenite showed increase in the expression for 3 metal (*mtl-1,* $p = 0.03$) and oxidative stress response (*cyp-35A2*, $p = 0.04$; *sod-1,* $p < 0.001$) genes, whereas worms in arsenate showed an increase in the expression for 5 metal (*mtl-1,* $p = 0.02$; *mtl-2,* $p < 0.001$) and oxidative stress response (*cyp-35A2*, $p = 0.04$; *isp-1,* $p = 0.05$; *ctl-2,* $p = 0.04$). These results suggest both *E. coli* and *L. sphaericus* fed worms, exposure to arsenics have increased stress response gene expression, however the extent of the response is relatively higher in *L. sphaericus* fed worms. In general, *L. sphaericus* fed worms concomitantly had increased expression of genes involved in metal response and oxidative stress response compared to the *E. coli* fed counterparts.

3.3. L. sphaericus B1CDA diet reduced the accumulation of reactive oxygen species in C. elegans

ROS and the resultant oxidative stress response have an important function in the health and lifespan of organisms (Ristow and Schmeisser, 2011). To determine if *L. sphaericus* influenced oxidative stress in *C. elegans* by reducing ROS, accumulation of ROS was analyzed using the fluorescent dye CM-H2DCFDA. *E. coli* fed worms presented a higher fluorescence intensity (23.98 ± 5.43) associated with the accumulation of ROS than those fed *L. sphaericus* (15.09 ± 3.41) ($p < 0.001$) (Fig. 3). Similarly, the ROS accumulation was also reduced in the positive control (paraquat) in worms fed *L. sphaericus* in comparison to those fed *E. coli* (Fig. 3B, Supplementary Fig. S1).

Arsenic induced oxidative stress response is a known mechanism of metal toxicity that could lead to a reduced lifespan. To determine if *L. sphaericus* can influence metal-associated oxidative stress in *C. elegans* by altering the accumulation of ROS was assessed in worms fed either *E. coli* or *L. sphaericus* exposed to arsenite or arsenate. In comparison to the respective K-media controls, both *E. coli* OP50 and *L. sphaericus* fed worms in the presence of arsenite had a significant increase in ROS (28.46 ± 4.55, $p < 0.001$; 21.63 ± 4.77, $p < 0.001$). Interestingly, only *E. coli* fed worms subjected to arsenate had significant increase in ROS (30.55 ± 5.31, $p < 0.001$) (Fig. 3B, Supplementary Fig. S1). Furthermore, *L. sphaericus* reduced ROS accumulation with both arsenic exposures in comparison to the *E. coli* fed worms (Fig. 3B). These results suggest that *L. sphaericus* both reduced ROS accumulation in *C. elegans* as well as increased the expression of genes associated with protection against oxidative stress.

3.4. L. sphaericus B1CDA diet altered the effects of arsenic on genes associated with lifespan and immune response in C. elegans

To determine the possible molecular mechanisms of arsenic toxicity on the longer-living *L. sphaericus* fed worms, the expression of 15 genes belonging to lifespan and immunity were compared to *E. coli* fed worms. Of the genes evaluated, 7 genes not affected by either *L. sphaericus* or arsenic treatment were *skn-1, cep-1, daf-12, daf-16, tol-1, tir-1* and *lys-7* (Supplementary Table S3). *L. sphaericus* fed worms in K-media showed significant change in expression of 4 genes; 3 genes whose expression increased were *hif-1* ($p = 0.021$), *abf-2* ($p = 0.037$), and *clec-60* (21.8-fold, $p = 0.027$), whereas *pgp-5* ($p = 0.002$) decreased (Fig. 4A).

E. coli fed worms in arsenics showed a significant decrease in the expression of lifespan associated genes (age-*1,* $p = 0.034$; *sir-2.1* $p = 0.041$) and an increase in expression for the infection response gene, *pgp-5* ($p = 0.020$) (Fig. 4A). Whereas *C. elegans* fed *L. sphaericus* in the control K-media compared to those fed *L. sphaericus* in the presence of either arsenic exhibited a significant change in gene expression in 5 of the 15 genes analyzed (Fig. 3A, supplementary Table 4). Genes associated with lifespan (*hif-1,* $p = 0.006$) and immune response (*abf-2,* $p = 0.005$; *bar-1,* $p = 0.003$; *clec-60*, $p = 0.02$; lys-8 $p = 0.02$) were significantly decreased in worms fed arsenite pre-treated *L. sphaericus* (Fig. 3A). However, worms fed arsenate pre-treated *L. sphaericus* presented a decrease in expression of lifespan (*hif-1,* $p = 0.01$), and immune response genes (*lys-8,* $p = 0.03$) (Fig. 3A). Furthermore, in comparison to the *E. coli* fed worms in arsenics, *L. sphaericus* fed worms showed an increase in expression for *abf-2* ($p = 0.02$) and *clec-60* ($p = 0.01$) and decrease in expression of *pgp-5* ($p = 0.003$) involved in immune response (Fig. 3A). These results suggest that arsenic exposure affects both lifespan and infection response gene expression in *C. elegans* with both *E. coli* as well as *L. sphaericus* diets, however *L. sphaericus* fed worms show reduced infection response gene expression.

3.5. L. sphaericus diet alters C. elegans fat associated gene expression and fat accumulation

Diet is known to affect lipid metabolism, and this has an important role in animal lifespan (Reinke et al., 2010). Since the *L. sphaericus* diet improved *C. elegans* lifespan, expression of 15 genes associated with fat metabolism (Supplementary Table S4) and total fat accumulation were assessed in L4/pre-adults stage worms. Nile red and oil red O staining were used to estimate the intracellular lipids in *C. elegans* fed either *E. coli* or L. *sphaericus* in the presence of K-media controls or arsenic species.

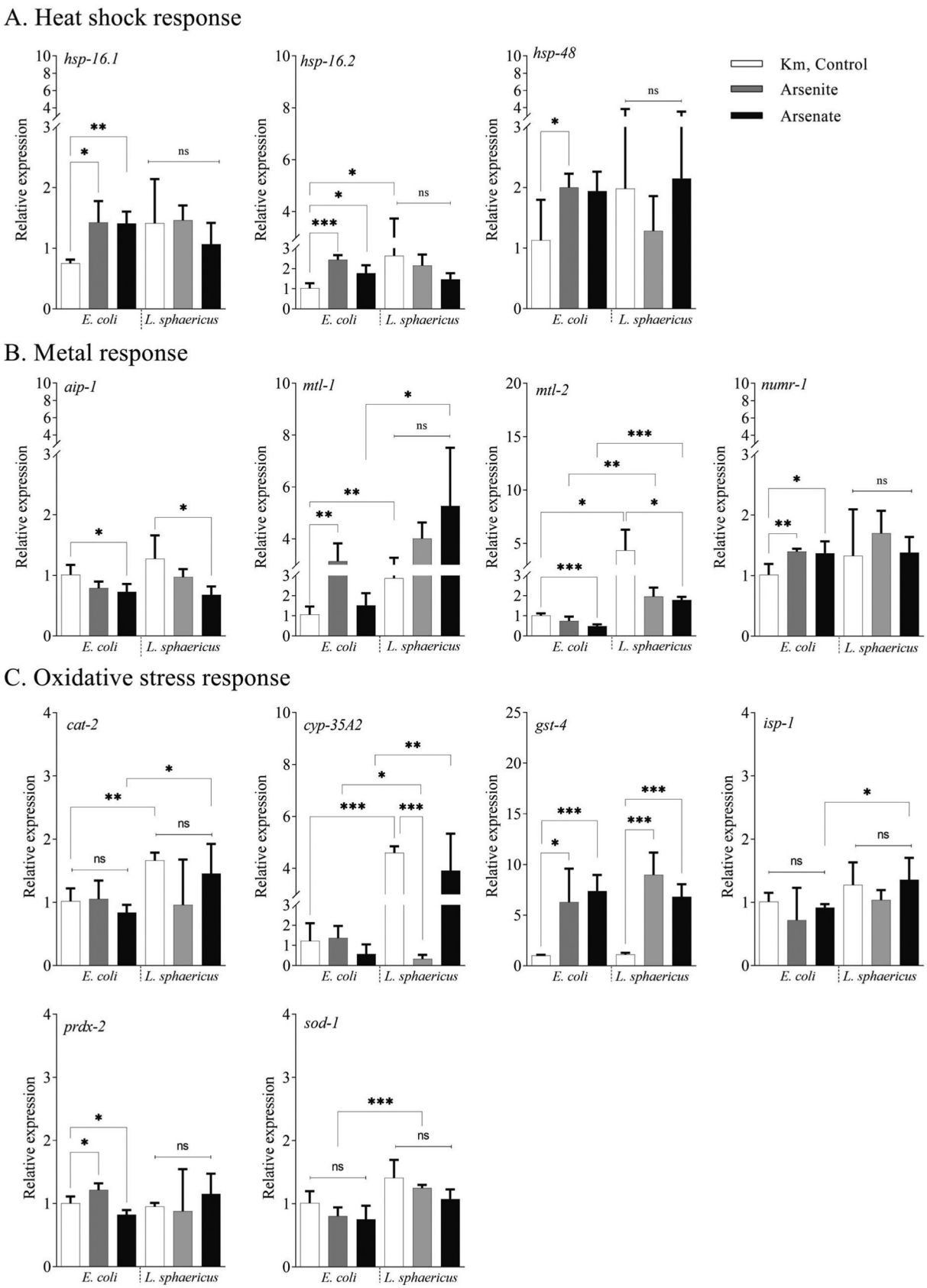


Fig. 2. Relative expression of genes associated with stress response in *C. elegans* fed *E. coli or L. sphaericus* exposed to arsenic species. Differential expression of genes belonging to (A) Heat shock (B) Metal response and (C) Oxidative stress response in *C. elegans* fed a diet of *E. coli* or *L. sphaericus* and exposed to arsenite (10 mg/L) or arsenate (10 mg/L). Data represent mean ± standard deviation from at least 4 biological replicates. Statistical significance was determined by two tailed unpaired *t*-test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p < 0.001$). *hsp-70* and *gcs-1* are not represented in the figure since the change in expression was not significant for any condition. Data are provided in supplementary Table S2.

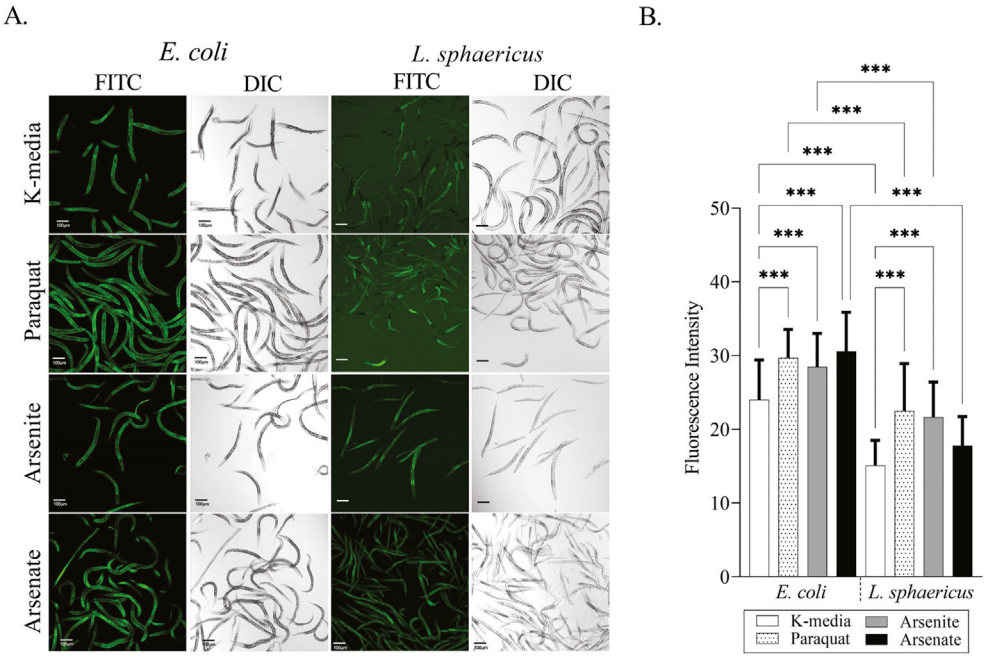


Fig. 3. Accumulation of reactive oxygen species in *C. elegans* fed *E. coli or L. sphaericus* exposed to arsenics. (A) Fluorescent micrographs of reactive oxygen species stained with CM-H2DCFDA and (B) quantification in *C. elegans* fed with *E. coli* OP50 or *L. sphaericus* B1CDA, when exposed to K-medium (control), paraquat (70 μM, positive control), arsenite (10 mg/L) or arsenate (10 mg/L). Scale bars represent 100 μm. Data represent the mean ± standard deviation from 4 independent experiments. Statistical significance was determined by one-way ANOVA followed by Tukeys multiple comparison test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p < 0.001$).

Relative to the *E. coli* K-media control *L. sphaericus* diet increased the expression of 2 genes, *fat-4* ($p = 0.006$) and *fat-7* ($p < 0.001$) (Fig. 4B), both involved in the polyunsaturated fatty acid synthesis, whereas expression was decreased in 4 genes involved in fatty acid biosynthesis, metabolism, and transport (*folt-2,* p = 0.006; *acl-6, p* = 0.01; *acly, p* = 0.04; and *vit-6,* p = 0.01), (Fig. 3B). In the fat accumulation assay using Nile red staining, *E. coli* showed a relative mean intensity of 31.19 ± 4.15, whereas in *L. sphaericus* fed worms the relative intensity was significantly reduced to 19.06 ± 3.09 (p < 0.001) (Fig. 5A and D, Supplementary Fig. S2 and Supplementary Table S4). Similarly, oil red O staining showed a significant decrease in staining intensity in *L. sphaericus* fed worms (36.14 ± 7.11) relative to the *E. coli* (58.48 ± 14.09) (Fig. 4B and E, Supplementary Fig. S2 and Supplementary Table S4). Furthermore, *L. sphaericus* fed worms showed no significant changes in body area to *E. coli* fed worms (Fig. 4C and supplementary Table S4). This suggests that *L. sphaericus* diet altered fat metabolism gene expression and concomitant decreased fat accumulation in *C. elegans.*

Humans and animals exposed to arsenics have alterations in fat metabolism (Garciafigueroa et al., 2013; Renu et al., 2018). Both *E. coli* and *L. sphaericus* fed worms exhibited differences in lifespan in the presence of arsenite or arsenate, therefore fat metabolism gene expression and fat accumulation were analyzed. Of the 15 genes evaluated, 2 genes not affected by either *L. sphaericus*, or arsenic treatment were *acs-5 and elo-2* (Supplementary Table S4). *E. coli* fed worms in arsenite presented a significant increase in the expression of 3 genes, two were associated with biosynthesis (*fat-5, p* = 0.03; *folt-2, p* = 0.04) and one associated with lipid metabolism (*acs-2, p* = 0.023) when compared to the *C. elegans* fed *E. coli* in K-media (Fig. 4B), whereas 6 genes had reduced expression, 2 associated with biosynthesis (*fat-6, p* = 0.018; *fasn-1, p* = 0.037) and 4 with lipid metabolism (*acl-4, p* = 0.029; *acl-6, p* = 0.002; *acs-11, p* = 0.017; *vit-6, p* = 0.015). Arsenate exposure of *E. coli* fed worms had increased expression of only one gene (*fat-5,* p = 0.02), and decreased expression of 8 genes analyzed (*acl-4, p* = 0.008; *acl-6,* p = 0.002; *acs-11, p* = 0.003; *acly,* p = 0.02; *vit-,6 p* = 0.013; *fat-6, p* < 0.001; *pod-2, p* = 0.012; *fasn-1,* p = 0.013) (Fig. 4B). Worms had a significant decrease in fat accumulation based on the relative intensity of Nile red when treated with either arsenite (20.98 ± 3.30; $p < 0.001$) or arsenate (22.91 ± 3.36; p < 0.001) (Fig. 5A and D, Supplementary Fig. S2), however oil red O as an alternative fat stain presented a significant decrease in fat accumulation only in arsenate fed worms (51.99 ± 14.38; p < 0.001) (Fig. 5B and E, Supplementary Fig. S2). Neither arsenite or arsenate exposure of *E. coli* fed worms altered body area (Fig. 5C).

L. sphaericus fed worms in the presence of arsenite or arsenate showed reduced expression of the *fat-6* (p < 0.001) and *fat-7* (p < 0.001) in comparison to the K-media control (Fig. 4B, Supplementary Table S2). Furthermore, *L. sphaericus* fed worms showed a reduced relative Nile red intensity in the presence of either arsenite (13.91 ± 2.72; p < 0.001) or arsenate (12.78 ± 2.90; p < 0.001) when compared to the control indicating reduced fat accumulation (Fig. 5A, D and Supplementary Fig. S2), whereas oil red O staining only showed a significant change in fat accumulation in arsenite fed worms (30.25 ± 9.80; $p < 0.01$) (Fig. 5B and E, Supplementary Fig. S2). A reduced body area was observed in *L. sphaericus* fed worms exposed to arsenite (0.84 ± 0.16; p < 0.001) and arsenate (0.88 ± 0.15; p < 0.001) in-comparison to the K-media controls (Fig. 4C). Taken together these results suggest that both arsenic species affect the expression of fat metabolism gene expression and accumulation of fat in worms despite the bacterial diet, however *L. sphaericus* diet appeared to have altered *C. elegans* fat metabolism gene expression associated with lipid synthesis and transport.

3.6. Multivariate analysis of gene expression showed association with bacterial food and arsenic exposure

Principal component analysis (PCA) was performed on the *C. elegans* gene expression data fed with either *E. coli* or *L. sphaericus* and exposed to K-media control or arsenics during the larvae stages. PCA model had an overall goodness of fit (R2X) value of 1.0 and goodness of prediction of (R2Q) value of 0.99, with the first two components explaining 70.5% of the variation. The principal components 1 (PC1) explained 37.2%, while the principal component 2 (PC2) explained 33.3% of the variation in the data (Fig. 6A). The first dimension separated the gene expression according to *E. coli* and L. *sphaericus* food source while the second dimension separated according to arsenic exposure. The loading plot (Fig. 6B) identified that most of the lifespan and immunity genes were associated with *L. sphaericus,* affecting *C. elegans* physiology and immunity. However, the strongest association for both food types were different immunity genes,

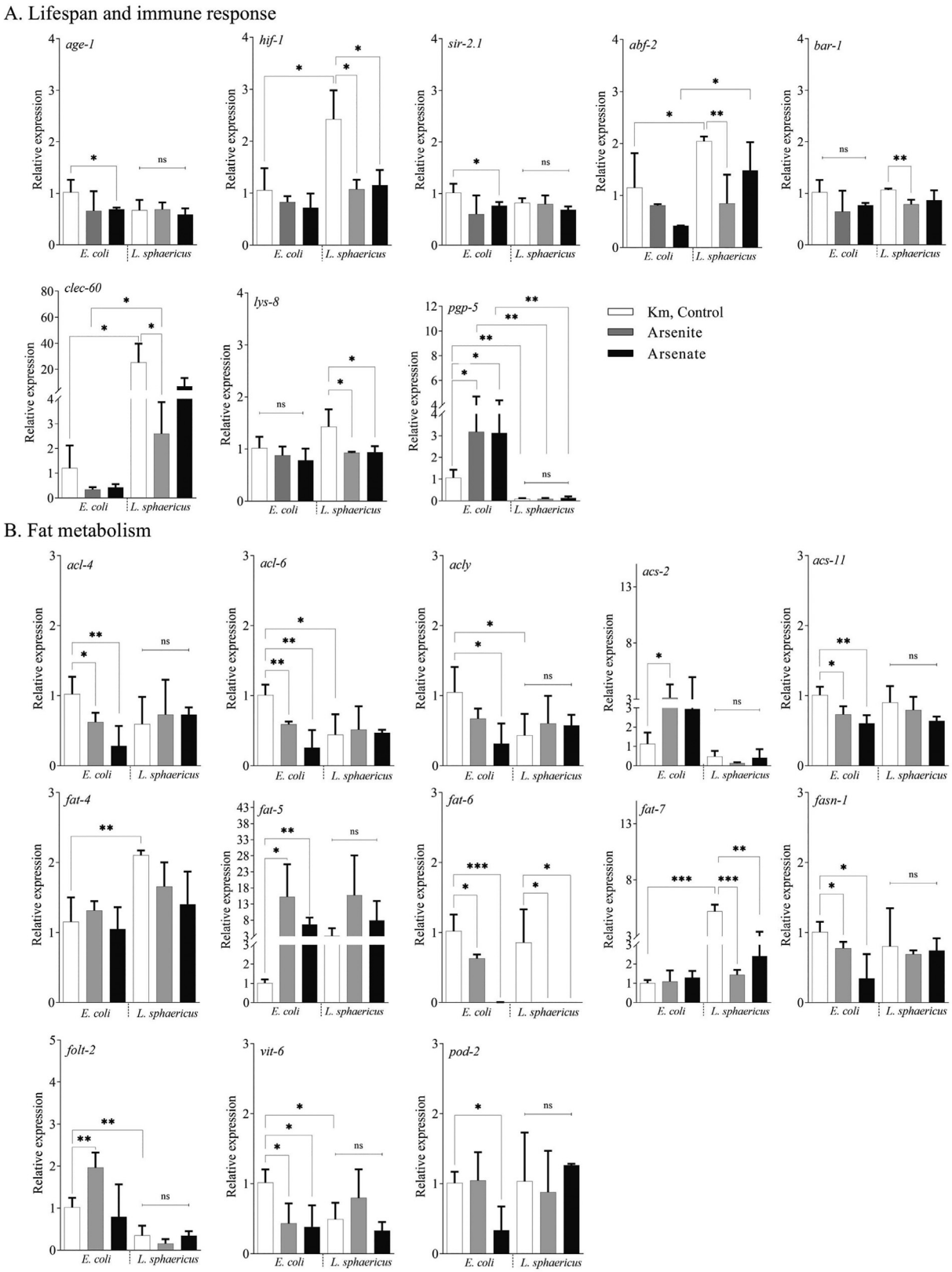


Fig. 4. Relative expression of genes associated with lifespan, immune response and fat metabolism in *C. elegans* fed *E. coli or L. sphaericus* treated with arsenics. Differential expression of genes associated with (A) lifespan and immune response and (B) fat metabolism in *C. elegans* upon exposure to *E. coli* or *L. sphaericus* diet in K-media (control), arsenite (10 mg/L) and arsenate (10 mg/L) when compared to *E. coli* K-media control. Data represents mean ± standard deviation of fold change in gene expression from at least 4 biological replicates. Two tailed unpaired t-test was used to establish statistical significance (*p ≤ 0.05; **p ≤ 0.01; ***p < 0.001). *cep-1, daf-12, daf-16, skn-1, tol-1, tir-1, lys-7* and *acl-5* were not significantly changed and are not represented in the figure. Data are provided in supplementary Tables S3 and S4.

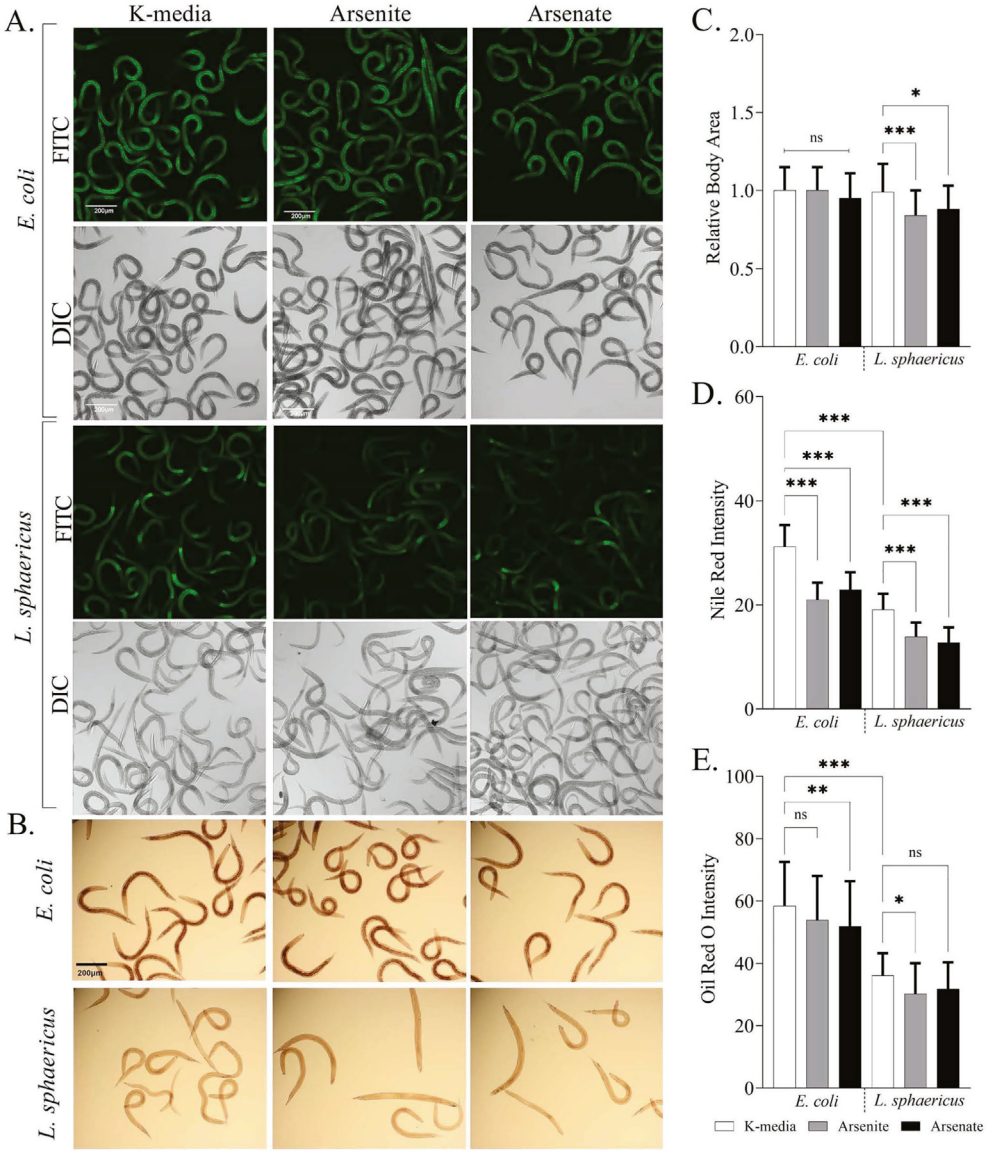


Fig. 5. Fat accumulation and body area in *C. elegans* fed bacteria exposed to arsenics. Micrographs of *C. elegans* stained for stored lipids with (A) Nile Red and (B) oil red O after being fed with *E. coli* OP50 or *L. sphaericus* B1CDA exposed to K-medium (control), arsenite (10 mg/L) or arsenate (10 mg/L). The scale bar represents 200 μm. Quantification of *C. elegans* (C) body area, (D) Nile Red intensity and (E) Oil Red O intensity of microscopy images. Data represent the mean ± standard deviation from at least 3 independent experiments. Statistical significance was determined by (C, D, E) one-way ANOVA followed by Tukeys multiple comparison test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p < 0.001$).

where *L. sphaericus* had the strongest association with *clec-60* (C-type LECtin-60), while *E. coli* had the strongest association with *pgp-5* (P-glycoprotein related-5). The response to arsenic differed according to the food source with the metal protective genes, *mtl-1* (Metallothionein-1) and *numr-1* (Nuclear localized metal responsive-1), were associated with *L. sphaericus* and the oxidative stress response gene, *gst-4* (Glutathione S-transferase-4), was associated with *E. coli*, while the remaining genes were common to both. The hierarchical cluster analysis (Fig. 6C) showed 3 distinct groupings, with food groups clustering together and arsenic treatment resulting in separate and distinct clustering. In addition, the effects of arsenic exposure were more pronounced in the presence of *E. coli* than *L. sphaericus*.

4. Discussion

Microbes act as an interface between the host and environment, therefore they should have an important role in mediating the host's response to xenobiotic substances. Chronic exposure to arsenics is a global problem and has been implicated in various physiological diseases and cancer, therefore it is important to increase our understanding of how microbes can alter host response to toxic metals. The present study showed that *C. elegans* were more resilient to arsenic when fed the arsenic-tolerant *L. sphaericus* B1CDA than the standard *E. coli* OP50 food. This was evidenced through the significantly longer lifespan and distinct physiological and genetic responses to arsenic when fed *L. sphaericus*.

The significant extension in lifespan in *C. elegans* fed *L. sphaericus* B1CDA compared to *E. coli* OP50 corroborates previous observations, indicating that the microbial food source impacts *C. elegans* fitness (Go et al., 2014). Previous studies have also reported that arsenic influences lifespan in *C. elegans* fed the standard *E. coli* OP50 food (Liao and Yu, 2005; Schmeisser et al., 2013; Yu et al., 2016). Similarly, *L. sphaericus* fed worms treated with arsenite or arsenate had a reduced lifespan, nevertheless, they still survived significantly longer than their untreated *E. coli* fed counter parts (Fig. 1). Several candidate probiotic bacterial species were previously shown to alter metal and chemical toxicants exposure directly through detoxification and indirectly through modulation of the host

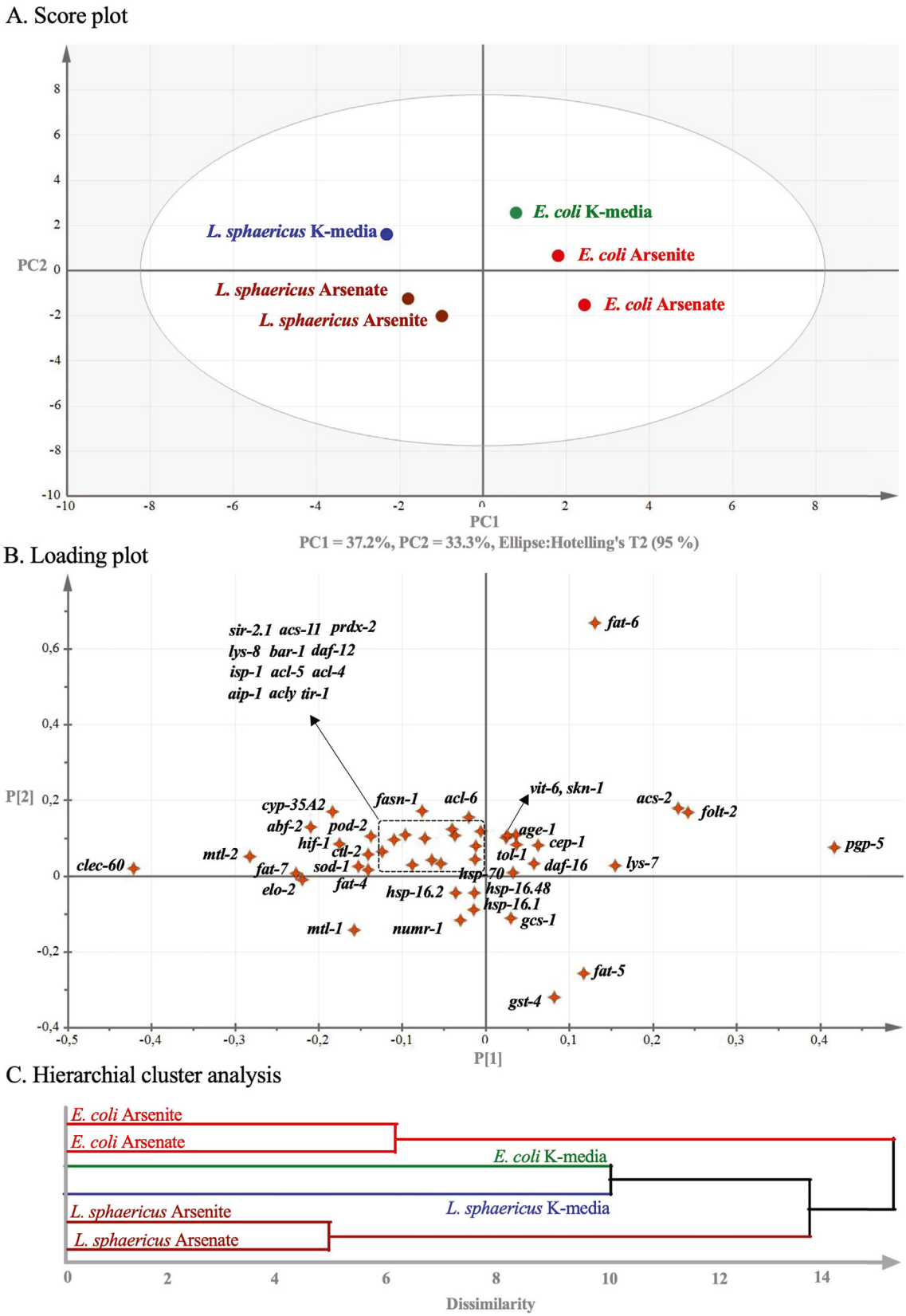


Fig. 6. Multivariate analysis of the *C. elegans* gene expression under the various conditions. Principal component analysis of the *C. elegans* gene expression responses in *E. coli* and *L. sphaericus* diets in the presence and absence of arsenics. (A) Score plot indicating 70.5% of the variation in the data are explained by the first 2 principal components (PC1 and PC2), (B) loading plot presenting the distribution of the genes in the different groups and (C) hierarchical cluster analysis based on the dissimilarity demonstrating the connectivity of the *C. elegans* gene expression responses with different diets and arsenic treatment.

responses (Kamaladevi et al., 2016; Zanjani et al., 2017). This prompted the hypothesis that the arsenic tolerant *L. sphaericus* B1CDA could protect *C. elegans* from arsenic toxicity and extend lifespan.

Metal-induced stress response is part of the cellular detoxification system and a primary indicator of the arsenic toxicity (Flora and Pachauri, 2013). In response to arsenite and arsenate exposures, *E. coli* fed worms showed an increased expression of several markers of arsenic toxicity, particularly genes involved in heat shock (*hsp-16.1, hsp-16.2, hsp-48*), metal (*mtl-1, numr-1*), oxidative stress (*gst-4, prdx-2*), as well as the accumulation of ROS (Fig. 2). These are consistent with the previous studies of arsenic and metal toxicity in *C. elegans* (Sahu et al., 2013; Yu et al., 2016). An exception to the previous studies was the downregulation of arsenic inducible protein (*aip-1),* which was previously shown to be upregulated in the presence of arsenic, however these studies used higher metal concentrations (Sahu et al., 2013). Heat shock proteins and metallothionein's play an important role in cellular stress response, homeostasis and are implicated in disease and development (Nielsen et al., 2007; Swindell, 2011). In *C. elegans* upregulation of *hsp-16* molecular chaperones were shown to extend lifespan (Mendenhall et al., 2012; Walker and Lithgow, 2003).

L. sphaericus fed worms showed increased expression of fewer genes implicated in stress responses, importantly heat shock (*hsp-16.2),* metal response (*mtl-1, mtl-2),* oxidative stress (*ctl-2*, *cyp-35A2)*, compared to *E. coli* fed worms, which could be associated with the accompanying decrease in the accumulation of ROS. Arsenic exposure in *L. sphaericus* fed worms also showed fewer changes in the expression of metal response (*mtl-1, mtl-2*) and oxidative stress response biomarker (*gst-4*), and higher levels of ROS were only present for arsenite treatment. Overall, the *L. sphaericus* fed worms had significantly lower levels of ROS than the *E. coli* fed worms, which likely contributes to the altered gene responses. Moreover, relative to the *E. coli* counterparts, *L. sphaericus* fed worms in arsenics showed increased gene expression for the metal (*mtl-1, mtl-2*), oxidative stress and detoxification response (*isp-1, ctl-2* and *sod-1*) (Fig. 2). Colonization of soil bacteria and biofilm formation of commensal bacteria in *C. elegans* intestine is implicated in increasing tolerance to stress, and increase in lifespan involving *mtl-1* and *mtl-2* (Coolon et al., 2009; Smolentseva et al., 2017). According to the free radical theory of aging, uncontrolled production of ROS coupled with an imbalance in antioxidant detoxification leads to increased cellular damage and this influences the progression of aging and disease (Vatner et al., 2020). Oxidative stress response detoxification genes, catalase and cytochrome monooxygenase perform several metabolic functions and are necessary for the detoxification of ROS, maintenance of fat metabolism and lifespan. Depletion of *ctl-2* or *cyp-35* were shown to affect both lifespan and fat accumulation in worms (Imanikia et al., 2015; Petriv and Rachubinski, 2004). Previous studies have shown that some strains of *Lactobacillus* and *Bifidobacterium* decrease the accumulation of ROS in worms and enhancing the nematode lifespan, primarily through increased antioxidant response and decreased lipid metabolism (Jin et al., 2020; Martorell et al., 2016). Since several stress response signatures upregulated with *L. sphaericus* B1CDA serves in improving cellular homeostasis in response to stress, it is likely that arsenic induced cellular stress is also reduced through increased antioxidative and cytoprotective mechanisms by the microbe.

Exposure to arsenics in humans as well as in animal models have been shown to affect development, lifespan, immune responses and fat metabolism (Carlson and Van Beneden, 2014; Farkhondeh et al., 2019). This suggests that microbes can influence the expression of genes involved in lifespan, immune response, fat metabolism and fat accumulation in *C. elegans* exposed to arsenic. In *E. coli* fed worms a decreased expression of genes associated with lifespan (*age-1*, *sir-2.1*) and increased expression of infection response gene *pgp-5* were observed in arsenite and arsenate exposures, (Fig. 3A). Both heavy metals and bacterial infections in *C. elegans* have been shown to upregulate *pgp-5* through TIR-1 and p38 MAP kinase pathways (Kurz et al., 2007), thus a combination of these challenges could explain the strong correlation to both *E. coli* diet and arsenic treatment (Fig. 6). An exception with the previous studies, is the downregulation of *daf-16,* though we observed a slight but not significant decrease in expression for *daf-16* (supplementary Table 2) as reported earlier (Yu et al., 2016).

L. sphaericus fed worms showed an increased expression of genes associated with lifespan (*hif-1)* and immunity (*abf-2 and clec-60)*, whereas arsenic exposure showed a decresed expression of these genes as well as *lys-8* and *bar-1*. When compared to the *E. coli* exposed to arsenics, *L. sphaericus* fed worms also showed differences in expression in *abf-2* and *clec-60,* genes encoding for defensin molecules (Fig. 3A, Supplimentary Table S3). Low oxygen environment induces activation of the conserved transcription factor, hypoxia-inducible factor (*hif-1*). HIF-1 alters cellular responses that mediate immunity and longevity in-concert with other regulators of aging (Leiser and Kaeberlein, 2010). In *C. elegans*, both over expression and loss-of-function of *hif-1* was shown to influence stress resistance and lifespan, and is mediated by other transcription factors (Zhang et al., 2009). Moreover long-lived AGE-1 mutants or pre-treatment of the AGE-1 mutants with the beneficial bacterium, *Lactobacillus zeae* were shown to have an increased expression of the defensin molecules (*abf-2* and *clec-60*) (Zhou et al., 2018). Although reduced expression of the p53 homologue (*cep-1*) was shown to improve nematode lifespan, it was not significantly altered in the present study (Arum and Johnson, 2007). These observations suggest that *L. sphaericus* helps preserve *C. elegans* immune function and thus contribute to extended lifespan even in the presence of arsenics.

Lipid metabolism is important in stress resistance and thus lifespan (Hou and Taubert, 2012). Exposure to toxic metals such as arsenics in humans and animal models have been shown to affect fat metabolism (Carlson and Van Beneden, 2014; Farkhondeh et al., 2019). Therefore, an altered bacterial diet could influence *C. elegans* lipid metabolism and by extension response to arsenic. *L. sphaericus* diet increased expression of *fat-4* and *fat-7,* genes involved in polyunsaturated fatty acid synthesis, whereas *folt-2, acl-6, acly* and *vit-6*, genes associated with fatty acid synthesis, metabolism, and transport had reduced expression when compared to *E. coli* diet (Fig. 3B). Arsenic exposed worms fed *L. sphaericus* diet affected the overall expression of fewer genes, primarily those involved in lipid synthesis (*fat-6, fat-7*) rather than lipid metabolism and transport. Several probiotic strains were previously shown to decrease lipid accumulation and modulate fat metabolism while enhancing the nematode host lifespan (Martorell et al., 2016). Studies with long lived *daf-2* and *age-1* mutants showed increased expression of genes involved in polyunsaturated fatty acid synthesis (*fat-7*), which are suggested to increase resistance to stress (Horikawa and Sakamoto, 2010; Murphy et al., 2003b; Shmookler Reis et al., 2011). Furthermore, RNAi inhibition of fat genes (*fat-6, fat-7, pod-2, fasn-1*) were shown to alter both resistance to stress and affect the survival of the worms (Dancy et al., 2015; Horikawa and Sakamoto, 2009; Zhang et al., 2013).

In addition to the changes in gene expression, both Nile red and oil red O staining showed *L. sphaericus* reduced the accumulation of lipids when compared to *E. coli* fed worms (Fig. 4A-D). Subsequent exposure to arsenics resulted in decreased fat accumulation in worms fed either bacterium using Nile red, and although it was not significant for oil red O staining, a downward trend was also observed (Fig. 4A-D). Nile red and oil red O staining were demonstrated to stain homeostasis lipids and triacylglycerols, respectively (Horikawa and Sakamoto, 2010). Nile red serves as a quantative technique for analysis of lipids, whereas oil red O is used for qualitative analysis of fat distribution and is considered unreliable for quantification of lipid levels (Escorcia et al., 2018). Studies in model organisms have shown altered fat metabolism in response to the arsenics (Farkhondeh et al., 2019), and recent studies also highlighted arsenic role in the decreased lipid accumulation as well as affected fat metabolism in monoculture and animal studies (Afolabi et al., 2015; Carlson and Van Beneden, 2014; Song et al., 2017; Zdraljevic et al., 2019; Zuo et al., 2019). Taken together, gene expression and lipid accumulation in the present study demonstrate that both bacteria and arsenic contribute to the modulation of host fat metabolism.

In summary the multivariate analysis of the gene expression data revealed that the main factor influencing *C. elegans* response was according to the bacterial food, while arsenic exposure was secondary. It showed

that *L. sphaericus* primarily influenced the immune response and fat metabolism to increase fitness and extend lifespan, indicating that host fitness is of primary importance in increasing resilience to toxic challenges. Arsenic exposure induced different stress responses which is likely due to the lower ROS levels in *C. elegans* fed *L. sphaericus,* suggesting that the bacteria contributed with its own detoxification mechanisms to reduce arsenic toxicity on the host. Taken together, the arsenic-tolerant and accumulating *L. sphaericus* contributed to the extended *C. elegans* lifespan though a multifactorial mechanism; by increasing worm fitness, while concomitantly decreasing arsenic toxicity and ROS exposure. This highly simplified model illustrated the role of food (nutrition) on host fitness as well as emphasises the role microbes have in detoxification of environmental contaminants. More complex models would provide furhter insight into how microbial interaction with each other and the host influences xenobiotic toxicity of contaminats such as arsenic.

CRediT authorship contribution statement

Jagadish C K Mangu: Methodology, Validation, Investigation, Formal analysis, Writing-Original Draft. **Neha Rai:** Methodology, Validation, Formal analysis, Writing - Review. **Abul Mandal:** Conceptualization, Writing-Review & Editing, Supervision. **Per-Erik Olsson:** Conceptualization, Resources, Writing-Review & Editing, Funding acquisition. **Jana Jass:** Conceptualization, Resources, Methodology, Validation, Investigation, Formal analysis, Writing-Original Draft, Review & Editing, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155377.

References

Afolabi, O.K., Wusu, A.D., Ogunrinola, O.O., Abam, E.O., Babayemi, D.O., Dosumu, O.A., et al., 2015. Arsenic-induced dyslipidemia in male albino rats: comparison between trivalent and pentavalent inorganic arsenic in drinking water. BMC Pharmacol. Toxicol. 16, 15.

Argos, M., 2015. Arsenic exposure and epigenetic alterations: recent findings based on the illumina 450K DNA methylation Array. Curr. Environ Health Rep. 2, 137–144.

Arum, O., Johnson, T.E., 2007. Reduced expression of the Caenorhabditis elegans p53 ortholog cep-1 results in increased longevity. J. Gerontol. A Biol. Sci. Med. Sci. 62, 951–959.

Babich, H., Stotzky, G., 1985. Heavy metal toxicity to microbe-mediated ecologic processes: a review and potential application to regulatory policies. Environ. Res. 36, 111–137.

Bellamri, N., Morzadec, C., Fardel, O., Vernhet, L., 2018. Arsenic and the immune system. Curr. Opin. Toxicol. 10, 60–68.

Ben Fekih, I., Zhang, C., Li, Y.P., Zhao, Y., Alwathnani, H.A., Saquib, Q., et al., 2018. Distribution of arsenic resistance genes in prokaryotes. Front. Microbiol. 9 2473-2473.

Bhattacharjee, P., Chatterjee, D., Singh, K.K., Giri, A.K., 2013. Systems biology approaches to evaluate arsenic toxicity and carcinogenicity: an overview. Int. J. Hyg. Environ. Health 216, 574–586.

Carlson, P., Van Beneden, R.J., 2014. Arsenic exposure alters expression of cell cycle and lipid metabolism genes in the liver of adult zebrafish (Danio rerio). Aquat. Toxicol. 153, 66–72.

Chi, L., Bian, X., Gao, B., Tu, P., Ru, H., Lu, K., 2017. The effects of an environmentally relevant level of arsenic on the gut microbiome and its functional metagenome. Toxicol. Sci. 160, 193–204.

Choiniere, J., Wang, L., 2016. Exposure to inorganic arsenic can lead to gut microbe perturbations and hepatocellular carcinoma. Acta Pharm. Sin. B 6, 426–429.

Coolon, J.D., Jones, K.L., Todd, T.C., Carr, B.C., Herman, M.A., 2009. Caenorhabditis elegans genomic response to soil bacteria predicts environment-specific genetic effects on life history traits. PLoS Genet. 5, e1000503.

Coryell, M., McAlpine, M., Pinkham, N.V., McDermott, T.R., Walk, S.T., 2018. The gut microbiome is required for full protection against acute arsenic toxicity in mouse models. Nat. Commun. 9, 5424.

Cubadda, F., Jackson, B.P., Cottingham, K.L., Van Horne, Y.O., Kurzius-Spencer, M., 2017. Human exposure to dietary inorganic arsenic and other arsenic species: state of knowledge, gaps and uncertainties. Sci. Total Environ. 579, 1228–1239.

Dancy, B.C.R., Chen, S.-W., Drechsler, R., Gafken, P.R., Olsen, C.P., 2015. 13C- and 15N-labeling strategies combined with mass spectrometry comprehensively quantify phospholipid dynamics in C. Elegans. PLoS One 10, e0141850.

Dong, X., Shulzhenko, N., Lemaitre, J., Greer, R.L., Peremyslova, K., Quamruzzaman, Q., et al., 2017. Arsenic exposure and intestinal microbiota in children from Sirajdikhan, Bangladesh. PLoS One 12, e0188487.

Druwe, I.L., Vaillancourt, R.R., 2010. Influence of arsenate and arsenite on signal transduction pathways: an update. Arch. Toxicol. 84, 585–596.

Escorcia, W., Ruter, D.L., Nhan, J., Curran, S.P., 2018. Quantification of lipid abundance and evaluation of lipid distribution in Caenorhabditis elegans by Nile red and oil red O staining. J. Vis. Exp. 133, e57352.

Farkhondeh, T., Samarghandian, S., Azimi-Nezhad, M., 2019. The role of arsenic in obesity and diabetes. J. Cell. Physiol. 234, 12516–12529.

Flora, S.J.S., Pachauri, V., 2013. Arsenic, free radical and oxidative stress. In: Kretsinger, R.H., Uversky, V.N., Permyakov, E.A. (Eds.), Encyclopedia of Metalloproteins. Springer, New York, New York, NY, pp. 149–159.

Gamble, M.V., Liu, X., Ahsan, H., Pilsner, R., Ilievski, V., Slavkovich, V., et al., 2005. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. Environ. Health Perspect. 113, 1683–1688.

Garciafigueroa, D.Y., Klei, L.R., Ambrosio, F., Barchowsky, A., 2013. Arsenic-stimulated lipolysis and adipose remodeling is mediated by G-protein-coupled receptors. Toxicol. Sci. 134, 335–344.

Giller, K.E., Witter, E., McGrath, S.P., 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. 30, 1389–1414.

Go, J., Lee, K.M., Park, Y., Yoon, S.S., 2014. Extended longevity and robust early-stage development of Caenorhabditis elegans by a soil microbe, Lysinibacillus sphaericus. Environ. Microbiol. Rep. 6, 730–737.

Gokulan, K., Arnold, M.G., Jensen, J., Vanlandingham, M., Twaddle, N.C., Doerge, D.R., et al., 2018. Exposure to arsenite in CD-1 mice during juvenile and adult stages: effects on intestinal microbiota and gut-associated immune status. MBio 9.

Gundert-Remy, U., Damm, G., Foth, H., Freyberger, A., Gebel, T., Golka, K., et al., 2015. High exposure to inorganic arsenic by food: the need for risk reduction. Arch. Toxicol. 89, 2219–2227.

Horikawa, M., Sakamoto, K., 2009. Fatty-acid metabolism is involved in stress-resistance mechanisms of Caenorhabditis elegans. Biochem. Biophys. Res. Commun. 390, 1402–1407.

Horikawa, M., Sakamoto, K., 2010. Polyunsaturated fatty acids are involved in regulatory mechanism of fatty acid homeostasis via daf-2/insulin signaling in Caenorhabditis elegans. Mol. Cell. Endocrinol. 323, 183–192.

Hou, N.S., Taubert, S., 2012. Function and regulation of lipid biology in Caenorhabditis elegans aging. Front. Physiol. 3, 143.

Imanikia, S., Hylands, P., Sturzenbaum, S.R., 2015. The double mutation of cytochrome P450's and fatty acid desaturases affect lipid regulation and longevity in C. Elegans. Biochem. Biophys. Rep. 2, 172–178.

Jin, X., He, Y., Liu, Z., Zhou, Y., Chen, X., Wang, G., et al., 2020. Lactic acid bacteria exhibit similar antioxidant capacities in Caenorhabditis elegans- and campylobacter jejuni-infected mice. RSC Adv. 10, 3329–3342.

Kaletta, T., Hengartner, M.O., 2006. Finding function in novel targets: C. Elegans as a model organism. Nat. Rev. Drug Discov. 5, 387–398.

Kamaladevi, A., Ganguli, A., Balamurugan, K., 2016. Lactobacillus casei stimulates phase-II detoxification system and rescues malathion-induced physiological impairments in Caenorhabditis elegans. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 179, 19–28.

Kumar, R., Pradhan, A., Khan, F.A., Lindström, P., Ragnvaldsson, D., Ivarsson, P., et al., 2015. Comparative analysis of stress induced gene expression in Caenorhabditis elegans following exposure to environmental and lab reconstituted complex metal mixture. PLoS One 10, e0132896.

Kurz, C.L., Shapira, M., Chen, K., Baillie, D.L., Tan, M.W., 2007. Caenorhabditis elegans pgp-5 is involved in resistance to bacterial infection and heavy metal and its regulation requires TIR-1 and a p38 map kinase cascade. Biochem. Biophys. Res. Commun. 363, 438–443.

Leiser, S.F., Kaeberlein, M., 2010. The hypoxia-inducible factor HIF-1 functions as both a positive and negative modulator of aging. Biol. Chem. 391, 1131–1137.

Liao, V.H., Yu, C.W., 2005. Caenorhabditis elegans gcs-1 confers resistance to arsenic-induced oxidative stress. Biometals 18, 519–528.

Lionaki, E., Tavernarakis, N., 2013. High-throughput and longitudinal analysis of aging and senescent decline in Caenorhabditis elegans. Methods Mol. Biol. 965, 485–500.

Lu, K., Abo, R.P., Schlieper, K.A., Graffam, M.E., Levine, S., Wishnok, J.S., et al., 2014. Arsenic exposure perturbs the gut microbiome and its metabolic profile in mice: an integrated metagenomics and metabolomics analysis. Environ. Health Perspect. 122, 284–291.

Martorell, P., Llopis, S., González, N., Chenoll, E., López-Carreras, N., Aleixandre, A., et al., 2016. Probiotic strain bifidobacterium animalis subsp. Lactis CECT 8145 reduces fat content and modulates lipid metabolism and antioxidant response in Caenorhabditis elegans. J. Agric. Food Chem. 64, 3462–3472.

Mendenhall, A.R., Tedesco, P.M., Taylor, L.D., Lowe, A., Cypser, J.R., Johnson, T.E., 2012. Expression of a single-copy hsp-16.2 reporter predicts life span. J. Gerontol. A Biol. Sci. Med. Sci. 67, 726–733.

Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., et al., 2003a. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 424, 277–283.

Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., et al., 2003b. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 424, 277–283.

Naujokas, M.F., Anderson, B., Ahsan, H., Aposhian, H.V., Graziano, J.H., Thompson, C., et al., 2013. The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. Environ. Health Perspect. 121, 295–302.

Nielsen, A.E., Bohr, A., Penkowa, M., 2007. The balance between life and death of cells: roles of metallothioneins. Biomark. Insights 1, 99–111.

Petriv, O.I., Rachubinski, R.A., 2004. Lack of peroxisomal catalase causes a progeric phenotype in Caenorhabditis elegans. J. Biol. Chem. 279, 19996–20001.

Pinyayev, T.S., Kohan, M.J., Herbin-Davis, K., Creed, J.T., Thomas, D.J., 2011. Preabsorptive metabolism of sodium arsenate by anaerobic microbiota of mouse cecum forms a variety of methylated and thiolated arsenicals. Chem. Res. Toxicol. 24, 475–477.

Prabhakaran, P., Ashraf, M.A., Aqma, W.S., 2016. Microbial stress response to heavy metals in the environment. RSC Adv. 6, 109862–109877.

Rahman, A., Nahar, N., Nawani, N.N., Jass, J., Desale, P., Kapadnis, B.P., et al., 2014. Isolation and characterization of a lysinibacillus strain B1-CDA showing potential for bioremediation of arsenics from contaminated water. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 49, 1349–1360.

Rahman, A., Nahar, N., Jass, J., Olsson, B., Mandal, A., 2016. Complete genome sequence of lysinibacillus sphaericus B1-CDA, a bacterium that accumulates arsenic. Genome Announc. 4.

Rai, N., Sjöberg, V., Forsberg, G., Karlsson, S., Olsson, P.E., Jass, J., 2019. Metal contaminated soil leachates from an art glass factory elicit stress response, alter fatty acid metabolism and reduce lifespan in Caenorhabditis elegans. Sci. Total Environ. 651, 2218–2227.

Reinke, S.N., Hu, X., Sykes, B.D., Lemire, B.D., 2010. Caenorhabditis elegans diet significantly affects metabolic profile, mitochondrial DNA levels, lifespan and brood size. Mol. Genet. Metab. 100, 274–282.

Renu, K., Madhyastha, H., Madhyastha, R., Maruyama, M., Arunachlam, S., GA, V., 2018. Role of arsenic exposure in adipose tissue dysfunction and its possible implication in diabetes pathophysiology. Toxicol. Lett. 284, 86–95.

Richardson, J.B., Dancy, B.C.R., Horton, C.L., Lee, Y.S., Madejczyk, M.S., Xu, Z.Z., et al., 2018. Exposure to toxic metals triggers unique responses from the rat gut microbiota. Sci. Rep. 8, 6578.

Ristow, M., Schmeisser, S., 2011. Extending life span by increasing oxidative stress. Free Radic. Biol. Med. 51, 327–336.

Sahu, S.N., Lewis, J., Patel, I., Bozdag, S., Lee, J.H., Sprando, R., et al., 2013. Genomic analysis of stress response against arsenic in Caenorhabditis elegans. PLoS One 8, e66431.

Schlebusch, C.M., Gattepaille, L.M., Engström, K., Vahter, M., Jakobsson, M., Broberg, K., 2015. Human adaptation to arsenic-rich environments. Mol. Biol. Evol. 32, 1544–1555.

Schmeisser, S., Schmeisser, K., Weimer, S., Groth, M., Priebe, S., Fazius, E., et al., 2013. Mitochondrial hormesis links low-dose arsenite exposure to lifespan extension. Aging (Albany NY) 12, 508–517.

Schulenburg, H., Félix, M.A., 2017. The natural biotic environment of Caenorhabditis elegans. Genetics 206, 55–86.

Shmookler Reis, R.J., Xu, L., Lee, H., Chae, M., Thaden, J.J., Bharill, P., 2011. Modulation of lipid biosynthesis contributes to stress resistance and longevity of C. elegans mutants. Aging (Albany NY) 3, 125–147.

Smolentseva, O., Gusarov, I., Gautier, L., Shamovsky, I., DeFrancesco, A.S., Losick, R., et al., 2017. Mechanism of biofilm-mediated stress resistance and lifespan extension in C. Elegans. Sci. Rep. 7, 7137.

Song, X., Li, Y., Liu, J., Ji, X., Zhao, L., Wei, Y., 2017. Changes in serum adiponectin in mice chronically exposed to inorganic arsenic in drinking water. Biol. Trace Elem. Res. 179, 140–147.

Stiernagle, T., 2006. Maintenance of C. Elegans. WormBook 1–11.

Swindell, W.R., 2011. Metallothionein and the biology of aging. Ageing Res. Rev. 10, 132–145.

Tchounwou, P.B., Yedjou, C.G., Udensi, U.K., Pacurari, M., Stevens, J.J., Patlolla, A.K., et al., 2019. State of the science review of the health effects of inorganic arsenic: perspectives for future research. Environ. Toxicol. 34, 188–202.

Thabit, S., Handoussa, H., Roxo, M., Cestari de Azevedo, B., El Sayed N, S.E., Wink, M., 2019. Styphnolobium japonicum (L.) schott fruits increase stress resistance and exert antioxidant properties in Caenorhabditis elegans and mouse models. Molecules 24, 2633.

Uno, M., Nishida, E., 2016. Lifespan-regulating genes in C. Elegans. NPJ Aging Mech. Dis. 2, 16010.

Van de Wiele, T., Gallawa, C.M., Kubachka, K.M., Creed, J.T., Basta, N., Dayton, E.A., et al., 2010. Arsenic metabolism by human gut microbiota upon in vitro digestion of contaminated soils. Environ. Health Perspect. 118, 1004–1009.

Vatner, S.F., Zhang, J., Oydanich, M., Berkman, T., Naftalovich, R., Vatner, D.E., 2020. Healthful aging mediated by inhibition of oxidative stress. Ageing Res. Rev. 64, 101194.

Walker, G.A., Lithgow, G.J., 2003. Lifespan extension in C. Elegans by a molecular chaperone dependent upon insulin-like signals. Aging (Albany NY) 2, 131–139.

Yan, G., Chen, X., Du, S., Deng, Z., Wang, L., Chen, S., 2019. Genetic mechanisms of arsenic detoxification and metabolism in bacteria. Curr. Genet. 65, 329–338.

Yu, C.W., How, C.M., Liao, V.H., 2016. Arsenite exposure accelerates aging process regulated by the transcription factor DAF-16/FOXO in Caenorhabditis elegans. Chemosphere 150, 632–638.

Zanjani, S.Y., Eskandari, M.R., Kamali, K., Mohseni, M., 2017. The effect of probiotic bacteria (Lactobacillus acidophilus and bifidobacterium lactis) on the accumulation of lead in rat brains. Environ. Sci. Pollut. Res. Int. 24, 1700–1705.

Zdraljevic, S., Fox, B.W., Strand, C., Panda, O., Tenjo, F.J., Brady, S.C., et al., 2019. Natural variation in C. Elegans arsenic toxicity is explained by differences in branched chain amino acid metabolism. elife 8, e40260.

Zhang, Y., Shao, Z., Zhai, Z., Shen, C., Powell-Coffman, J.A., 2009. The HIF-1 hypoxia-inducible factor modulates lifespan in C. Elegans. PLoS One 4, e6348.

Zhang, Y., Zou, X., Ding, Y., Wang, H., Wu, X., Liang, B., 2013. Comparative genomics and functional study of lipid metabolic genes in Caenorhabditis elegans. BMC Genomics 14, 164.

Zhang, J., Holdorf, A.D., Walhout, A.J.C., 2017. Elegans and its bacterial diet as a model for systems-level understanding of host-microbiota interactions. Curr. Opin. Biotechnol. 46, 74–80.

Zhou, M., Liu, X., Yu, H., Yin, X., Nie, S.P., Xie, M.Y., et al., 2018. Cell signaling of Caenorhabditis elegans in response to enterotoxigenic Escherichia coli infection and lactobacillus zeae protection. Front. Immunol. 9, 1745.

Zuo, Z., Liu, Z., Gao, T., Yin, Y., Wang, Z., Hou, Y., et al., 2019. Prolonged inorganic arsenic exposure via drinking water impairs brown adipose tissue function in mice. Sci. Total Environ. 668, 310–317.