Heliyon

*Received:*
2 March 2018
*Revised:*
20 September 2018
*Accepted:*
3 January 2019

Cite as: Ramona Dölling, Martha N. Mendelski, Rüdiger J. Paul. Bacterial diet and weak cadmium stress affect the survivability of *Caenorhabditis elegans* and its resistance to severe stress. Heliyon 5 (2019) e01126. doi: 10.1016/j.heliyon.2019.e01126

# Bacterial diet and weak cadmium stress affect the survivability of *Caenorhabditis elegans* and its resistance to severe stress

**Ramona Dölling, Martha N. Mendelski, Rüdiger J. Paul***

*Institute of Zoophysiology, University of Münster (WWU), 48143, Münster, Germany*

* Corresponding author.

E-mail address: paulr@uni-muenster.de (R.J. Paul).

## Abstract

Stress may have negative or positive effects in dependence of its intensity (hormesis). We studied this phenomenon in *Caenorhabditis elegans* by applying weak or severe abiotic (cadmium, $CdCl_2$) and/or biotic stress (different bacterial diets) during cultivation/breeding of the worms and determining their developmental speed or survival and performing transcriptome profiling and RT-qPCR analyses to explore the genetic basis of the detected phenotypic differences. To specify weak or severe stress, developmental speed was measured at different cadmium concentrations, and survival assays were carried out on different bacterial species as feed for the worms. These studies showed that 0.1 $\mu$mol/L or 10 mmol/L of $CdCl_2$ were weak or severe abiotic stressors, and that *E. coli* HT115 or *Chitinophaga arvensicola* feeding can be considered as weak or severe biotic stress. Extensive phenotypic studies on wild type (WT) and different signaling mutants (e.g., *kgb-1*Δ and *pmk-1*Δ) and genetic studies on WT revealed, inter alia, the following results. WT worms bred on *E. coli* OP50, which is a known cause of high lipid levels in the worms, showed high resistance to severe abiotic stress and elevated gene expression for protein biosynthesis. WT worms bred under weak biotic stress (*E. coli* HT115 feeding

https://doi.org/10.1016/j.heliyon.2019.e01126

which causes lower lipid levels) showed an elevated resistance to severe biotic stress, elevated gene expression for the innate immune response and signaling but reduced gene expression for protein biosynthesis. WT worms bred under weak biotic and abiotic stress (*E. coli* HT115 feeding plus 0.1 $\mu$mol/L of $CdCl_2$) showed high resistance to severe biotic stress, elevated expression of DAF-16 target genes (e.g., genes for small heat shock proteins) but further reduced gene expression for protein biosynthesis. WT worms bred under weak biotic but higher abiotic stress (*E. coli* HT115 feeding plus 10 $\mu$mol/L of $CdCl_2$) showed re-intensified gene expression for the innate immune response, signaling, and protein biosynthesis, which, however, did not caused a higher resistance to severe biotic stress. *E. coli* OP50 feeding as well as weak abiotic and biotic stress during incubations also improved the age-specific survival probability of adult WT worms. Thus, this study showed that a bacterial diet resulting in higher levels of energy resources in the worms (*E. coli* OP50 feeding) or weak abiotic and biotic stress promote the resistance to severe abiotic or biotic stress and the age-specific survival probability of WT.

Keywords: Cell biology, Molecular biology, Toxicology, Physiology

## 1. Introduction

Hormesis describes the intensity-dependent positive or negative effects of several stressors [1]. Low concentrations of the heavy metal copper cause, for instance, a higher reproduction rate in *Campanularia flexuosa* [2], and low quantities of polyphenol tannic acid (TA) have positive effects on the thermal tolerance and oxidative stress resistance of the model organism *C. elegans* [3]. Low concentrations of the heavy metal cadmium promote nitrate reductase activity in *Hydrilla verticillata* [4] but high cadmium concentrations may result in oxidative stress, protein damage, and disturbed DNA repair [5, 6]. Resistance mechanisms to severe cadmium stress include the antioxidative defense, for instance, by the glutathione redox system, protection and repair of proteins by heat shock proteins, binding of cadmium by metallothioneins, and export of cadmium by ATP binding cassette (ABC) transporters and associated proteins (glutathione, phytochelatin) [7, 8, 9].

The bacterial diet of *C. elegans* also bears the risk of ingesting pathogenic bacteria. However, feeding *C. elegans* with the pathogenic *E. coli* strain 536 revealed a hormetic effect (i.e., higher thermal tolerance due to the expression of small heat shock proteins; [10]). Even *E. coli* OP50 as standard bacterial diet of *C. elegans* in the laboratory [11] has been reported to be mildly pathogenic [12], especially for older worms [13, 14]. *E. coli* HT115 [15] as another bacterial diet of *C. elegans* in the laboratory (primarily for RNA interference assays; [16]) differs, for instance, from *E. coli* OP50 by a longer outer core of the lipopolysaccharides (LPS) in the outer


https://doi.org/10.1016/j.heliyon.2019.e01126

membrane [17, 18, 19], which was reported to affect positively the life span of *C. elegans* wild type (WT) worms at a higher cultivation temperature (25 °C; [20]). However, Brooks et al. [21] did not find appreciable effects of different *E. coli* strains (including *E. coli* HT115) on the life span of WT at 20 °C, with a slightly higher maximum life span on *E. coli* OP50. These authors as well as Maier et al. [20] and Xiao et al. [22] showed a higher fat storage (triacylglycerol level) in WT on *E. coli* OP50 than on *E. coli* HT115. The feeding rate of WT was also higher on *E. coli* OP50 than on *E. coli* HT115 at 20 °C [22] and 25 °C [20]. The developmental speed of WT was reported to be either higher (at 25 °C; [20]) or lower (at 20 °C; [22]) on *E. coli* OP50 than on *E. coli* HT115. WT also produced a higher number of progeny on *E. coli* OP50 than on *E. coli* HT115 (at 25 °C; [20]). Thus, these two bacterial diets evoke substantial differences in the metabolism, behavior, development, and reproduction of WT, which seem to vary with temperature.

Important signaling and processing units for the cellular responses of *C. elegans* to abiotic or biotic stress are the PMK-1 and KGB-1 mitogen-activated protein kinase (MAPK) and DAF-2 insulin-like signaling pathways. The p38-type PMK-1 MAPK pathway promotes the heat or cadmium resistance of *C. elegans* [23, 24], with PMK-1 promoting in the latter case also DAF-16-mediated gene expression. PMK-1 signaling is also important for the innate immune response [25, 26, 27] by promoting the expression of immune response genes. PMK-1 target genes encode C-type lectins, CUB-like domain proteins, and ShK toxins [26]. The antimicrobial effect of C-type lectins was proven in mice [28] but *C. elegans* also showed an upregulation of several C-type lectins under pathogenic stress [29]. Even if possible functions or roles in pathogenic defense mechanisms of the CUB-like domain proteins and metridin-like ShK toxins are still under debate [26, 30, 31], an upregulation of corresponding genes was also detected in *C. elegans* under pathogenic stress [26]. The JNK-like KGB-1 MAPK pathway participates in the responses to heavy metal stress [32, 33, 34] via the transcription factor FOS-1 [35], and it is also involved in innate immune responses [25, 36]. Active DAF-2 signaling prevents the nuclear translocation of the transcription factor DAF-16. Inhibited DAF-2 signaling, however, results in the nuclear translocation of non-phosphorylated DAF-16 and DAF-16 target gene expression. These genes encode, for instance, small heat shock proteins (α-crystallins), metallothioneins (MTL-1/2), and antioxidant enzymes (superoxide dismutase 3) [37] or the lysozyme LYS-7 [26]. DAF-16 is also an essential life-prolonging transcription factor [38].

This study on *C. elegans* focused on the effects of weak abiotic and/or biotic stress on the age-specific survival probability and the resistance to severe abiotic or biotic stress. Weak as well as severe abiotic and biotic stressors were identified in developmental and survival assays. The effects of weak abiotic and/or biotic stress on the age-specific survival probability of adult wild type and two MAPK signaling mutants (*kgb-1*Δ, *pmk-1*Δ) were determined in long-term (over weeks) survival assays.


https://doi.org/10.1016/j.heliyon.2019.e01126

The effects of weak abiotic and/or biotic stress during breeding on the later resistance of adult wild type to severe abiotic or biotic stress were determined in short-term (over days) survival assays. To explore the genetic basis of the effects of weak abiotic and/or biotic stress, transcriptome analyses were performed by RNA-Seq on wild type worms, which were bred under weak abiotic and/or biotic stress conditions. Results of the RNA-Seq study were checked by quantitative RT-PCR analyses. The results of this study showed that either a bacterial diet causing a higher level of energy resources in the worms or the exposure to weak abiotic and biotic stress significantly promote the age-specific survival probability and the resistance to severe abiotic or biotic stress of wild type.

## 2. Materials and methods

### 2.1. Experimental organisms

*C. elegans* of the N2 Bristol variety (wild type, WT) and the signaling mutants *kgb-1*Δ [KB3 *kgb-1(um3)* IV], *pmk-1*Δ [KU25, *pmk-1(km25)* IV], *daf-2*Δ [CB1370, *daf-2(e1370)* III], and *daf-16*Δ [CF1038, *daf-16(mu86)* I] as well as the *Escherichia coli* strain OP50 were obtained from the *Caenorhabditis* Genetics Center (CGC) (https://www.cbs.umn.edu/research/resources/cgc). The *E. coli* strain HT115 (empty vector L4440) was provided by Source BioScience LifeSciences (Nottingham, UK). *Chitinophaga arvensicola* was obtained from the DSMZ (DSM No. 3695; Braunschweig, Germany). The maintenance of *C. elegans* and the composition of *C. elegans*-specific media and buffers followed the instructions of [11]. Worms were bred at 20 °C on NGM (nematode growth medium) plates (petri dishes, ∅ = 9.2 cm) seeded with 1 mL of *E. coli* OP50 suspension [$OD_{600nm}$ = 1; LB (lysogeny broth) or OP50 medium, with the latter containing 0.01 g/L uracil (Sigma-Aldrich, Germany)]. All experiments were carried out at T = 20 °C as well. According to the German law, experiments carried out on the invertebrate *C. elegans* do not have to be announced or approved.

### 2.2. Development studies

WT worms were synchronized, and ten eggs were transferred onto NGM plates (∅ = 6 cm) seeded with 1 mL of *E. coli* OP50 suspension ($OD_{600nm}$ = 1; OP50 medium) and containing cadmium chloride ($CdCl_2 \times 2½ H_2O$; Sigma-Aldrich, Germany) at final concentrations between 0 and 300 μmol/L. The number of worms of each developmental stage (L1, L2, L3, L4 larval stages, young adult and adult worms) was determined every day (always at the same time of day) by visual observation and counting.


https://doi.org/10.1016/j.heliyon.2019.e01126

## 2.3. Short-term survival assay

Synchronized L1 larvae of WT and the signaling mutants were transferred onto NGM plates (∅ = 9.2 cm) seeded with 1 mL of *E. coli* HT115 suspension ($OD_{600nm}$ = 1; LB medium). After reaching adulthood, worms were washed off the NGM plates with M9 buffer and transferred onto empty NGM plates to get rid of bacteria. Then, 15 adult worms were transferred onto NGM plates (∅ = 3.5 cm) seeded with 0.2 mL of *E. coli* OP50, *E. coli* HT115, or *C. arvensicola* suspension ($OD_{600nm}$ = 0.5; LB medium). Flight behavior was minimized by a thin layer of 10 mg/mL of palmitic acid (BioXtra, Sigma-Aldrich, Germany) around the edge of the petri dishes [39]. Survival was tested after 1, 2, 3, and 4 d (always at the same time of day) by applying weak mechanical stimuli to evoke body movements (touch response), with the worms transferred onto new NGM plates after 3 d to avoid a lack of food or overcrowding. Worms that had buried themselves into the NGM or climbed up the edge of the petri dishes were not considered.

## 2.4. Long-term survival assay

Synchronized L1 larvae of WT, *kgb-1*Δ, and *pmk-1*Δ were transferred onto NGM plates (∅ = 9.2 cm) seeded with 1 mL of *E. coli* HT115 suspension ($OD_{595nm}$ = 0.7; LB medium). The adult worms were washed off the NGM plates with M9 buffer and transferred onto empty NGM plates to remove bacteria. Then, 15 adult worms were transferred onto NGM plates (∅ = 6 cm) seeded with 0.5 mL of *E. coli* OP50 or *E. coli* HT115 suspension ($OD_{595nm}$ = 0.7; LB medium) and containing 0 or 0.1 μmol/L of $CdCl_2$. Flight behavior was minimized as described above. Survival was tested daily (always at the same time of day) by eliciting the touch response, with the worms transferred onto new NGM plates every second day. Worms within the NGM or on the edge of the petri dishes were not considered.

## 2.5. Resistance to severe abiotic or biotic stress after breeding on varied $CdCl_2$ concentrations and bacterial diets

Synchronized L1 larvae of WT were transferred onto NGM plates (∅ = 9.2 cm) seeded with 1 mL of *E. coli* OP50 or *E. coli* HT115 suspension ($OD_{600nm}$ = 1; LB medium) and containing 0 or 0.1 μmol/L of $CdCl_2$ for the abiotic stress (i.e., 10 mmol/L of $CdCl_2$) or 0, 0.1, or 10 μmol/L of $CdCl_2$ for the biotic stress (i.e., *C. arvensicola* feeding). Adult worms were washed off the NGM plates with M9 buffer and transferred onto empty NGM plates to get rid of bacteria. For testing the resistance to severe abiotic stress, 15 adult worms were transferred onto NGM plates (∅ = 6 cm) seeded with 0.5 mL of *E. coli* OP50 or HT115 suspension ($OD_{600nm}$ = 1; LB medium) and containing 0 or 10 mmol/L of $CdCl_2$. Survival was tested after 1 d by eliciting the touch response. For testing the resistance to severe biotic stress, 15 adult worms were transferred onto NGM plates (∅ = 3.5 cm)


https://doi.org/10.1016/j.heliyon.2019.e01126

seeded with 0.2 mL of *C. arvensicola* suspension ($OD_{600nm} = 0.5$; LB medium). Survival was tested after 4 d by eliciting the touch response, with the worms transferred onto new NGM plates after 3 d. Flight behavior was minimized in both cases as described above. Worms within the NGM or on the edge of the petri dishes were not considered.

## 2.6. RNA-Seq

Transcriptome profiling was carried out under five experimental conditions. WT worms were incubated for three weeks on NGM plates ($\varnothing = 9.2$ cm) seeded with 1 mL of *E. coli* (i) OP50 (abbr. OP50) or (ii) HT115 (abbr. HT115_1) suspension ($OD_{600nm} = 1$; LB medium) or on NGM plates seeded with 1 mL of *E. coli* HT115 suspension and containing (iii) 0 (abbr. HT115_2), (iv) 0.1 (abbr. 0.1 Cd), or (v) 10 (abbr. 10 Cd) $\mu$mol/L of $CdCl_2$. The worms were transferred onto new NGM plates twice a week. Subsequently, synchronized L1 larvae were transferred onto 15 different NGM plates ($\varnothing = 9.2$ cm) per experimental condition (see above). After reaching adulthood, worms were washed off from the NGM plates and cleaned several times using purified water to get rid of bacteria. Five plates per experimental condition were combined to one biological sample. Thus, three biological replicates per experimental condition were further prepared for RNA-Seq.

The following procedure has already been described in [23]. Briefly, RNAiso-G (Segenetic, Borken, Germany) was added to the samples, before they were frozen in liquid nitrogen. After thermal disruption of worms (liquid nitrogen, 35 °C; three repetitions), chloroform extraction on ice (10 min), and centrifugation (12.000 × *g*, 4 °C, 15 min), DNA was digested and RNA purified with a RNase-free DNase set and RNeasy® mini kit (Qiagen, Hilden, Germany). Quality control was performed using an Agilent Bioanalyzer® (Agilent Technologies, Böblingen, Germany). After adding the samples to RNAstable™ matrix (Biomatrica, San Diego, CA, USA) and a subsequent vacuum centrifugation for drying (2 h), the samples from three experimental approaches were sent to the Beijing Genomics Institute (BGI) for RNA-Seq analysis (OP50 and HT115_1; HT115_2 and 10 Cd; 0.1 Cd).

Using Illumina HiSeq2000 technology, samples were sequenced at the BGI with a minimum of 10 megareads per sample and a sequencing quality of more than 98% clean reads. Sequences were mapped to Wormbase release WS257. Mean expression intensities of genes (i.e., means from the three biological replicates per experimental condition, after checking for substantial deviations between corresponding data; see below) and differential gene expression were calculated with the FPKM method (fragments per kilobase of exon per million fragments mapped) to normalize for sequencing depth and gene length. The NOISeq method [40] was used to determine the threshold of *P* (diverge probability $\geq 0.8$) for the differentially expressed genes (DEGs). In case of OP50, HT115_2, 0.1 Cd, and 10 Cd, the three


https://doi.org/10.1016/j.heliyon.2019.e01126

biological replicates at each test condition showed a high degree of similarity with regard to the expression intensity of corresponding genes, and mean expression intensities were calculated using the data from all three biological replicates. In case of HT115_1, however, one biological replicate differed significantly from the two others regarding the expression intensity of corresponding genes, and mean expression intensities were calculated here using the data from only two biological replicates.

## 2.7. Quantitative reverse transcription PCR (RT-qPCR)

Samples for RNA-Seq (before matrix embedment) were also used for RT-qPCR analyses (see [24] for details). Briefly, RNA concentrations were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Darmstadt, Germany). After reverse transcription of 0.5 $\mu$g of total RNA per sample using oligo $(dT)_{18}$-primers (First Strand cDNA Synthesis Kit; Fermentas, St. Leon-Rot, Germany) and RevertAid Reverse Transcriptase (Thermo Fisher Scientific), quantitative PCR was performed using a Real-Time PCR System (Eco Real-Time PCR; Illumina) and the PerfeCTa SYBR® Green SuperMix Kit (Quanta Biosciences, Beverly, MA, USA). Each sample contained 1 $\mu$L of template cDNA, 5 $\mu$L of SYBR Green SuperMix, 1 $\mu$L of the specific primer pair (10 mmol/L each; see Table 1 for the sequences), and 3 $\mu$L of purified water, resulting in an end volume of 10 $\mu$L. Then, the following procedure was applied: 2 min at 95 °C (initial phase), 40 cycles in the order 1 min at 95 °C, 10 s at 95 °C; 30 s at 60 °C, followed by a melting curve (between 60 °C and 95 °C). Data analysis was accomplished via ΔCq calculations [41]. Ratios were calculated between the mRNA levels of the genes of interest and the housekeeping gene *cdc-42* [42].

**Table 1. Primer sequences used in qRT-PCR.** Primers were designed using the PerlPrimer tool [60].

| Gene ID | Public name | Forward primer | Reverse Primer |
|---|---|---|---|
| WBGene00021580 | *clec-174* | GTAACTACTTGGCAGTCTTCG | CTTGCCATTATCGTCCAGTG |
| WBGene00021224 | *clec-209* | CTTCTTAGCATCAATCGTTCGT | TTTGTGAAATCAGGTGTGGAC |
| WBGene00020945 | *clec-219* | AAGAAGAACCAACACCACCT | ACAGAAGCAAACATACACCAC |
| WBGene00018971 | *clec-67* | TGATGGTGACAGTTCAAAGC | TTCCAAAAATGCCCGAGTAG |
| WBGene00016577 | *clec-3* | ACTGTTCGATGTGAAGAATGC | GTAGCAGGGATCCTCATGAG |
| WBGene00013931 | *clec-97* | AGACTTGCGATACTGGATGG | TGATCCAGATTGATTACCAAGG |
| WBGene00008916 | *clec-221* | AATGGGATGATCAACTGACTG | AGGAAGTGAGCAATCTTTGTC |
| WBGene00008202 | *clec-197* | GTGCATCAAGGTTTACCCAG | TCTTCCACATATCTCTTCTCGG |
| WBGene00000390 | *cdc-42* | TTCGACAATTACGCCGTCAC | CCTGAGATCGACTTGAGTACC |


https://doi.org/10.1016/j.heliyon.2019.e01126

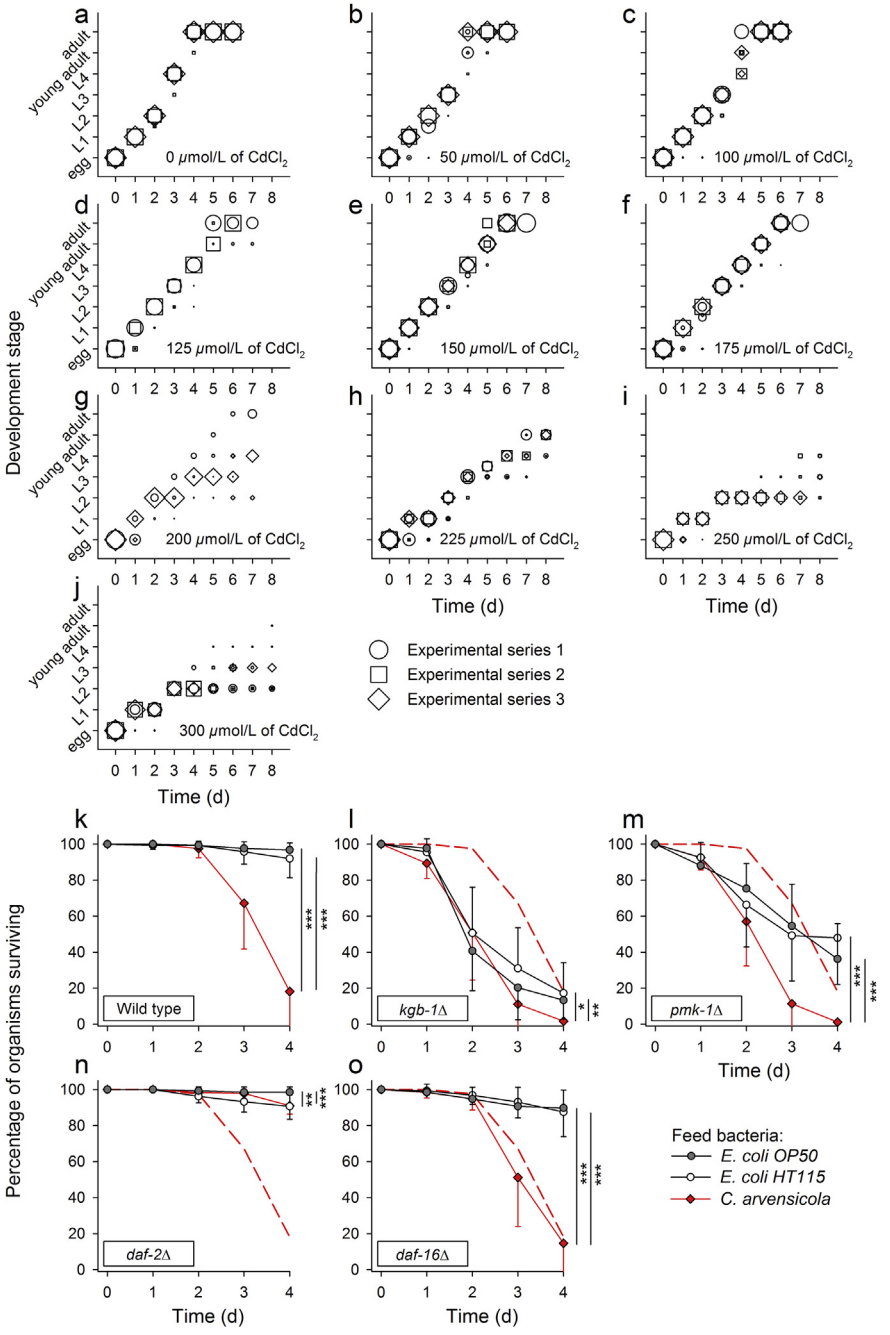


**Fig. 1.** The effects of different $CdCl_2$ concentrations or bacterial diets on the developmental speed or short-term survival probability of worms. The developmental speed of wild type from egg to adult worm was determined (a−j) at different $CdCl_2$ concentrations (0−300 μmol/L of $CdCl_2$) in the NGM plates at 20 °C, using *E. coli* OP50 as bacterial diet (per $CdCl_2$ concentration, $n = 3$ biological replicates on $N = 10$ worms each). The diameter of the symbols marks the relative number of worms of a specific developmental stage, which was counted from day 0 (egg deposition) to day 8. The diameter of the symbols at day 0 corresponds to 100% (i.e., 10 worms). The survival probability of synchronized adult (k) wild type, (l) *kgb-1*Δ, (m) *pmk-1*Δ, (n) *daf-2*Δ, and (o) *daf-16*Δ worms, which were fed with *E. coli* OP50 (gray circles), *E. coli* HT115 (white circles), or *C. arvensicola* (red diamonds), was determined


https://doi.org/10.1016/j.heliyon.2019.e01126

## 2.8. Bioinformatics

David Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/tools.jsp) was used for GO (gene ontology) analyses (i.e., functional annotation charts). Cluster analysis was carried out using the program Cluster 3.0 (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm). For this program, the common DEGs of the contrasts *E. coli* HT115 vs. *E. coli* OP50 feeding, 0.1 vs. 0 μmol/L of $CdCl_2$, and 10 vs. 0.1 μmol/L of $CdCl_2$, with *E. coli* HT115 as bacterial diet in the latter two cases, were initially determined. After that, gene clustering was performed, with the WormBase identification numbers (WB GeneIDs) and mean expression intensities of the 1607 common DEGs in the five differently treated worm groups (HT115_1, OP50, 0.1 Cd, 10 Cd, HT115_2; see above) as starting values. The data were $\log_2$-transformed, and the genes and arrays were separately centered (using the medians) and normalized. Hierarchical clustering of the genes and arrays was performed using the similarity metric 'correlation (uncentered)' and the clustering method 'average linkage'. The dendrogram was created with the program Java TreeView 1.1.6r4 (https://sourceforge.net/projects/jtreeview/).

## 2.9. Statistics and computations

Data are given as mean ± standard deviation (SD) or as mean of means ± standard error (SE). *n* indicates the number of biological replicates and *N* the number of animals used. SigmaPlot 11.0 (Systat Software, Erkrath, Germany) was used for graph preparations and statistical analysis. Two-way analysis of variance (ANOVA, including the Holm-Sidak all pairwise multiple comparison procedure) or t-tests were used to identify significant differences ($P \leq 0.05$, 0.01, or 0.001) in survival or mRNA level.

# 3. Results

## 3.1. Abiotic and biotic stress influence the developmental speed and short-term survival probability of worms

The developmental speed of wild type (WT) from egg to adult worm was studied on nematode growth medium (NGM) plates containing $CdCl_2$ at concentrations between 0 and 300 μmol/L (Fig. 1a–j). A $CdCl_2$ concentration of 50 μmol/L already induced a slight developmental delay (Fig. 1b) in comparison to the control (Fig. 1a). At $CdCl_2$ concentrations of more than 175 μmol/L, the worms rarely or never

over a period of four days at 20 °C (mean ± SD; per strain and bacterial diet, $n = 9–12$ biological replicates on $N = 15$ worms each). Dashed curves show the survival probability of wild type on *C. arvensicola* as bacterial diet (from k). Asterisks mark significant differences after four days of incubation ($^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $P^{***} \leq 0.001$; two-tailed t-test).

reached adulthood (Fig. 1g–j). These results also suggest that an exposure to a $CdCl_2$ concentration of 0.1 μmol/l causes only a weak abiotic stress.

Feeding synchronized adult wild type and the synchronized signaling mutants *kgb-1*Δ, *pmk-1*Δ, *daf-2*Δ, and *daf-16*Δ with *E. coli* OP50, *E. coli* HT115, or *C. arvensicola* revealed negative effects of *C. arvensicola* feeding on the survival probability after four days of incubation (Fig. 1k–o). The mutants *kgb-1*Δ (Fig. 1l) and *pmk-1*Δ (Fig. 1m) additionally showed a severely reduced survival probability in comparison to wild type (Fig. 1k) on any bacterial diet. The negative effect of *C. arvensicola* feeding was lowest in *daf-2*Δ, whose survival probability was maximum on *E. coli* OP50 (Fig. 1n). The survival probability of *daf-16*Δ (Fig. 1o) was slightly below that of wild type at any bacterial diet. These results suggest that *C. arvensicola* feeding causes a severe biotic stress, whereas *E. coli* HT115 feeding may represent a weak biotic stress in comparison to *E. coli* OP50 feeding.

## 3.2. Weak abiotic and/or biotic stress affect the age-specific survival probability of worms

Determining the survival probability of synchronized adult wild type (Fig. 2a–e), *kgb-1*Δ (Fig. 2f–j), and *pmk-1*Δ (Fig. 2k–o) at $CdCl_2$ concentrations of 0 or 0.1 μmol/L in the NGM and on *E. coli* OP50 or *E. coli* HT115 as bacterial diet revealed differential effects of these experimental conditions on the age-specific survival probability during the remaining life span of these strains. Although there were distinct strain-specific differences in life span, the different experimental conditions had no effect on maximum life span.

The age-specific survival probability of wild type worms was higher on *E. coli* OP50 than on *E. coli* HT115 (Fig. 2a) and on *E. coli* HT115 with 0.1 μmol/L of $CdCl_2$ in the NGM than on *E. coli* HT115 without $CdCl_2$ in the NGM (Fig. 2c). Conversely, the age-specific survival probability of *kgb-1*Δ worms was lower on *E. coli* OP50 than on *E. coli* HT115 (Fig. 2f). It was also lower on *E. coli* OP50 without or with 0.1 μmol/L of $CdCl_2$ in the NGM than on *E. coli* HT115 with 0.1 μmol/L of $CdCl_2$ in the NGM (Fig. 2i, j). The age-specific survival probability of *pmk-1*Δ worms was also lower on *E. coli* OP50 than on *E. coli* HT115 (Fig. 2k), but it was higher on *E. coli* OP50 without or with 0.1 μmol/L of $CdCl_2$ in the NGM than on *E. coli* HT115 with 0.1 μmol/L of $CdCl_2$ in the NGM (Fig. 2n, o). In contrast to wild type, *pmk-1*Δ showed a higher age-specific survival probability on *E. coli* HT115 (without $CdCl_2$ in the NGM) than on *E. coli* HT115 with 0.1 μmol/L of $CdCl_2$ in the NGM. Thus, the differences in cadmium exposure and/or bacterial diet caused quite different effects on the age-specific survival probability of WT, *kgb-1*Δ, or *pmk-1*Δ.


https://doi.org/10.1016/j.heliyon.2019.e01126

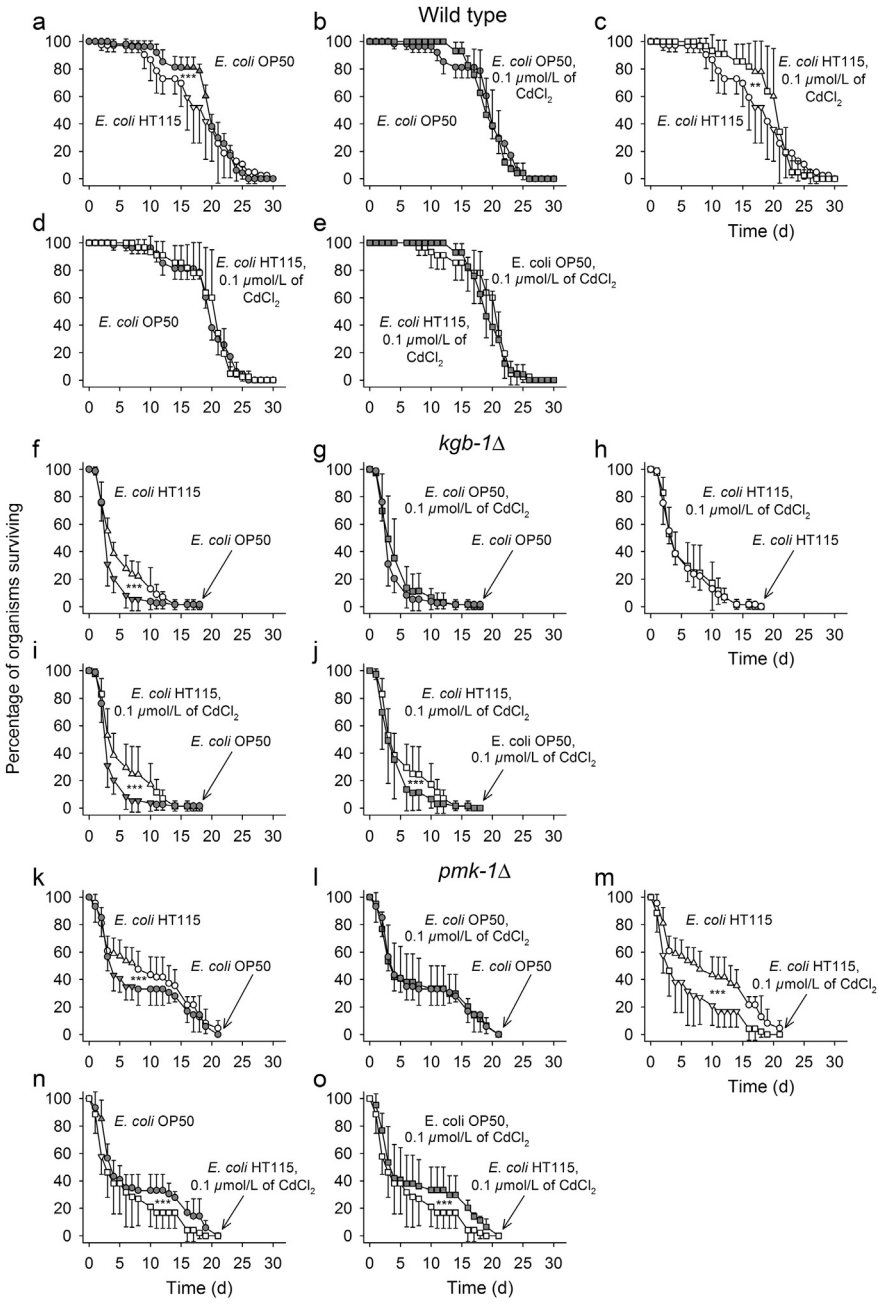


**Fig. 2.** $CdCl_2$ concentration and bacterial diet influence the age-specific survival probability of wild type, *kgb-1*Δ, and *pmk-1*Δ. The age-specific survival probability of synchronized adult (a−e) wild type, (f−j) *kgb-1*Δ, and (k−o) *pmk-1*Δ worms, which were incubated on NGM plates containing either no $CdCl_2$ (circles and/or triangles) or 0.1 μmol/L of $CdCl_2$ (squares and/or triangles) and fed with *E. coli* OP50 (gray symbols) or *E. coli* HT115 (white symbols) was determined over periods of up to 30 (wild type), 18 (*kgb-1*Δ) or 21 (*pmk-1*Δ) days at 20 °C (mean ± SD; per strain, $CdCl_2$ concentration, and bacterial diet, $n = 3-6$ biological replicates on $N = 15$ worms each). Asterisks mark significant differences between whole curves ($^{**}P \leq 0.01$; $P^{***} \leq 0.001$; two-way ANOVA), with triangle symbols indicating significant differences on specific days of incubation ($P \leq 0.05$; Holm-Sidak all pairwise multiple comparison procedure).


https://doi.org/10.1016/j.heliyon.2019.e01126

### 3.3. Breeding under weak abiotic and/or biotic stress influence the stress resistance of wild type

After breeding wild type from egg to adult worm at different $CdCl_2$ concentrations in the NGM (0, 0.1, or 10 $\mu$mol/L) on *E. coli* OP50 or *E. coli* HT115 as bacterial diet, the adult worms were exposed for one day to severe abiotic stress (10 mmol/L of $CdCl_2$, with the respective bacterial diet remaining unchanged) or for four days to severe biotic stress (*C. arvensicola* feeding). The resistance to severe abiotic stress was higher on *E. coli* OP50 than on *E. coli* HT115 but also higher after breeding at 0.1 $\mu$mol/L of $CdCl_2$ in the NGM in case of *E. coli* HT115 feeding (Fig. 3a). The resistance to severe biotic stress was maximum after breeding at 0.1 $\mu$mol/L of $CdCl_2$ and lower after breeding at 0 or 10 $\mu$mol/L of $CdCl_2$, with the positive effect of 0.1 $\mu$mol/L of $CdCl_2$ particularly strong after breeding on *E. coli* HT115 (Fig. 3b). Thus, differences in cadmium exposure and/or bacterial diet during breeding affected the stress resistance of WT.

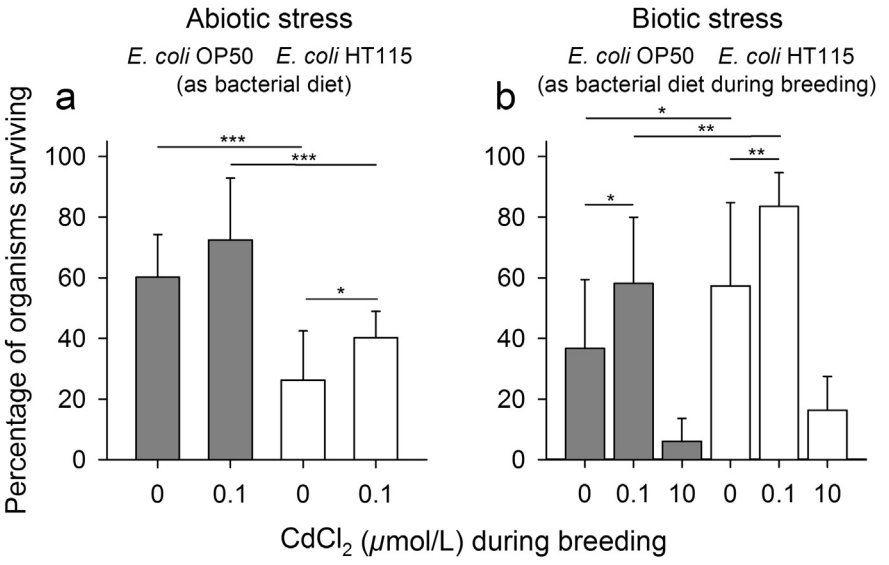


**Fig. 3.** Breeding on varied $CdCl_2$ concentrations and bacterial diets affect the resistance of wild type to severe abiotic or biotic stress. Wild type developed from egg to adult worm on NGM plates containing 0, 0.1, or 10 $\mu$mol/L of $CdCl_2$, with either *E. coli* OP50 (gray bars) or *E. coli* HT115 (white bars) as bacterial diet. Afterwards, the stress resistance of the adult worms (mean ± SD) was determined after (a) one day on NGM plates containing 10 mmol/L of $CdCl_2$ (*see* Fig. 1a–j), with the specific bacterial diet remaining unchanged (per $CdCl_2$ concentration and bacterial diet during breeding, $n = 9$ biological replicates on $N = 15$ worms each) or (b) four days with *C. arvensicola* as bacterial diet (*see* Fig. 1k–o) (per $CdCl_2$ concentration and bacterial diet during breeding, $n = 10–16$ biological replicates on $N = 15$ worms each) at 20 °C. Asterisks mark significant differences ($^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $P^{***} \leq 0.001$; two-tailed t-test).


https://doi.org/10.1016/j.heliyon.2019.e01126

### 3.4. Breeding under weak abiotic and/or biotic stress affect gene expression in wild type

To explore the genetic basis of these results, transcriptome profiling by RNA-Seq was performed on synchronized adult wild type worms. For this, worms were long-term incubated and bred with *E. coli* OP50 or *E. coli* HT115 as bacterial diet to study effects of the different feeding conditions or with 0, 0.1, or 10 $\mu$mol/L of $CdCl_2$ in the NGM and *E. coli* HT115 as bacterial diet to study effects of the different cadmium concentrations. More than 18,000 genes were identified and carried a WormBase identification number (WB GeneID). Genes termed as differentially expressed genes (DEGs) showed a diverge probability of greater than 0.8. To identify higher regulated genes or DEGs, the mean expression intensities (mean FPKM values) of corresponding genes or DEGs from different experimental conditions, which carried identical WB GeneIDs, were plotted against each other (x-y plots) (Fig. 4 and Supplementary Fig. S1). Higher regulated groups of genes or DEGs showed substantial deviations from the 45-degree diagonal line in double linear plots (left graphs) or distinct $\log_2$-fold differences in expression intensity in double $\log_2$ plots (right graphs), with the two scale types chosen to emphasize genes with higher (double linear plots) or lower expression intensity (double $\log_2$ plots). The function of these groups of genes or DEGs was studied by gene ontology (GO) analyses (functional annotation chart; David Bioinformatics Resources 6.8) (Table 2 and Supplementary Table S1).

The gene or DEG groups identified in the double linear plots (Figs. 4 and S1, left graphs, colored symbols) commonly comprised genes or DEGs for translational processes, with annotation terms like ribosomal protein or translation (Tables 2 and S1; terms marked with the Greek character α). Genes or DEGs for translational processes were negatively affected by *E. coli* HT115 feeding in comparison to *E. coli* OP50 feeding (Figs. 4a and S1a) and particularly by 0.1 $\mu$mol/L of $CdCl_2$ but also by 10 $\mu$mol/L of $CdCl_2$ in comparison with *E. coli* HT115 feeding without $CdCl_2$ in the NGM (Fig. 4c, e and S1c). Contrasting the two different cadmium concentrations showed that the expression of genes or DEGs for translational processes was more negatively affected by 0.1 than by 10 $\mu$mol/L of $CdCl_2$ (Figs. 4g and S1g).

The gene or DEG groups identified in the double $\log_2$ plots (Figs. 4 and S1, right graphs, colored symbols) comprised genes, which were frequently assigned to terms (*see* Tables 2 and S1, terms marked with Greek characters) that are likely related to innate immune response (β) (e.g., C-type lectin, CUB-like domain, metridin-like ShK toxin), sensory perception (γ) (e.g., detection of chemical stimulus, G-protein coupled receptor signaling, olfactory behavior, 7-TM GPCR), signaling (δ) (e.g., de-/phosphorylation), or stress response (ε) (e.g., α-crystallin, ER unfolded protein response). Genes or DEGs associated with innate immune response were upregulated on *E. coli* HT115 (Table 2: b; Table S1: b, g, h), *E. coli* OP50 (Table 2: c),


https://doi.org/10.1016/j.heliyon.2019.e01126

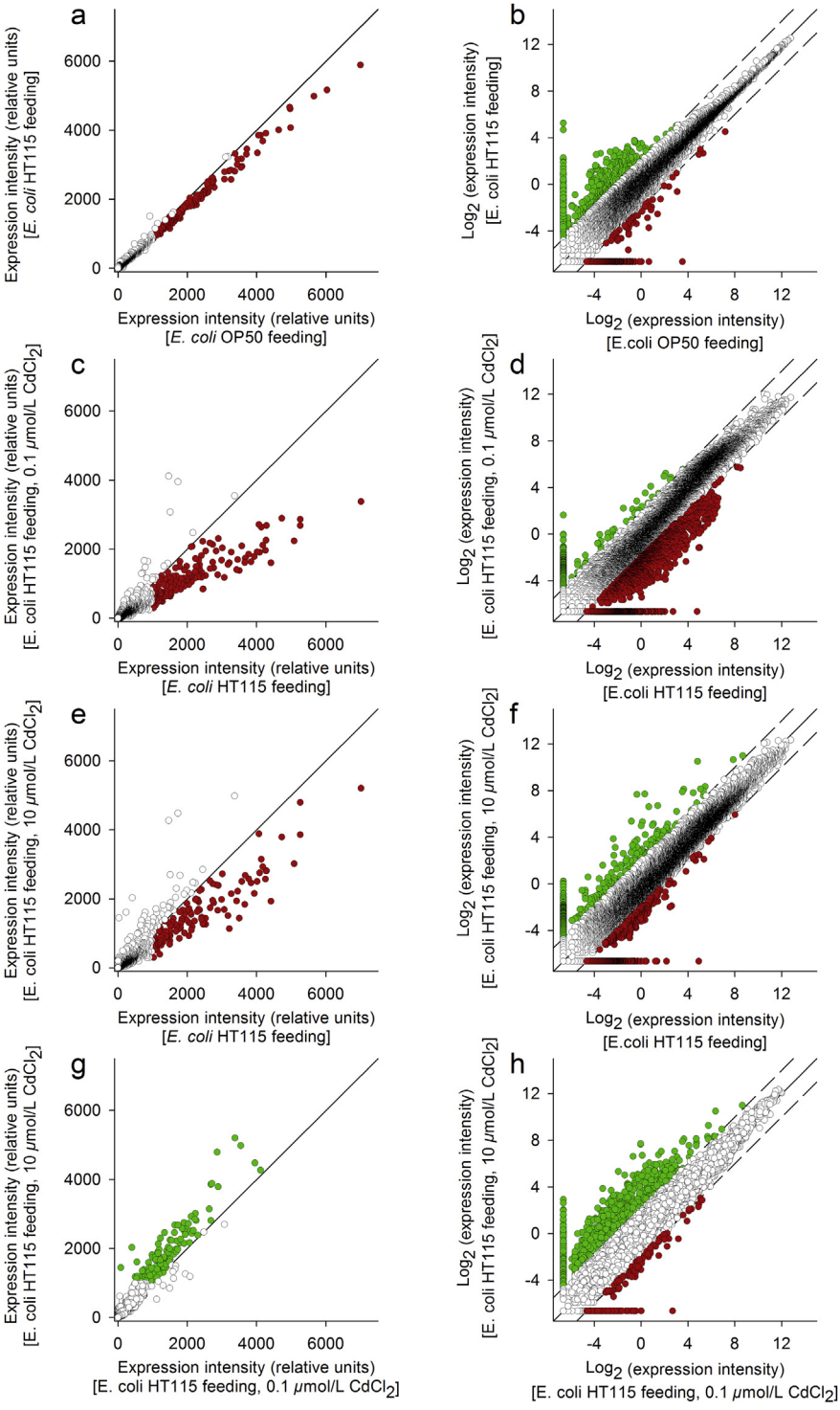


**Fig. 4.** Breeding on varied $CdCl_2$ concentrations and bacterial diets affect gene expression in wild type. Transcriptome profiling was performed by RNA-Seq to determine gene expression intensities in differently treated synchronized adult wild type worms (mean FPKM values; per $CdCl_2$ concentration and bacterial diet, $n = 2-3$ biological replicates, *see* Materials and methods). For this, an initial long-term incubation (for three weeks) and the subsequent development from egg to adult worm took place on


https://doi.org/10.1016/j.heliyon.2019.e01126

and *E. coli* HT115 with 10 $\mu$mol/L of $CdCl_2$ (Table 2: h, k; Table S1: i, l, m). Genes or DEGs associated with signaling were upregulated on *E. coli* HT115 (Table 2: f; Table S1: b, h) and *E. coli* HT115 with 10 $\mu$mol/L of $CdCl_2$ (Table 2: k; Table S1: l). Genes or DEGs associated with sensory perception were upregulated on *E. coli* HT115 (Table 2: b, i), *E. coli* OP50 (Table 2: c), and *E. coli* HT115 with 0.1 $\mu$mol/L of $CdCl_2$ (Table 2: e, l). Genes or DEGs associated with stress responses were upregulated on *E. coli* HT115 (Table S1: b, h), *E. coli* HT115 with 0.1 (Table 2: l; Table S1: e, o) or 10 $\mu$mol/L of $CdCl_2$ in the NGM (Table 2: h; Table S1: l).

Thus, genes or DEGs likely involved in the innate immune response or signaling were primarily upregulated on *E. coli* HT115 and *E. coli* HT115 with 10 $\mu$mol/L of $CdCl_2$. Genes or DEGs likely involved in sensory perception were never upregulated on *E. coli* HT115 with 10 $\mu$mol/L of $CdCl_2$. Genes or DEGs likely involved in the stress response were never upregulated on *E. coli* OP50. The most intensely upregulated DEGs underpinned this result (Fig. 5a–d). DEGs for the immune defense (C-type lectins, fungus-induced proteins) were frequent on *E. coli* HT115 (Fig. 5a) but also on *E. coli* HT115 with 10 $\mu$mol of $CdCl_2$ in the NGM (Fig. 5c, d). Stress genes such as DEGs for heat shock proteins (HSP-70, small heat shock proteins) or the cadmium defense (CDR-1, MTL-1/2) were more frequent on *E. coli* HT115 with 0.1 or 10 $\mu$mol of $CdCl_2$ in the NGM (Fig. 5b, c).

As transcriptome profiling frequently showed an upregulation of C-type lectins in the analyzed contrasts, we determined the relative expression level of several C-type lectin mRNAs by qRT-PCR using the respective samples for RNA-Seq. Actually, there was a significant upregulation of several C-type lectin mRNAs in worms exposed to 10 $\mu$mol/L of $CdCl_2$ in comparison to control worms, with *E. coli* HT115 feeding in both cases (Fig. 5e).

A cluster analysis on the 1607 common DEGs of five experimental conditions (HT115_1, OP50, 0.1 Cd, 10 Cd, HT115_2; *see* Materials and methods) was carried out (Supplementary Fig. S2). GO analyses on the DEG clusters a0-a22 and b0-b22

NGM plates (at 20 °C) containing either (a, b) no $CdCl_2$, with *E. coli* OP50 or *E. coli* HT115 as bacterial diet, or (c, d) 0 (control) or 0.1 $\mu$mol/L of $CdCl_2$, (e, f) 0 (control) or 10 $\mu$mol/L of $CdCl_2$, and (g, h) 0.1 $\mu$mol/L or 10 $\mu$mol/L of $CdCl_2$, with *E. coli* HT115 as bacterial diet in c–h. The x-y plots show the mean expression intensities of all genes, which carried a WormBase identification number (WB GeneID), under two different experimental conditions as double linear (left graphs) or double $\log_2$ (right graphs) plots to emphasize genes with higher or lower expression intensity. Highly regulated groups of genes with clearly different expression intensities under the two experimental conditions can be found in the gene clusters, which deviate substantially from the 45-degree diagonal line in the double linear plots and show expression intensities of greater than 1,000 (red or green circles) or differ in their $\log_2$-fold expression intensities by values of greater than 2 (red circles) or lesser than -2 (green circles) between control (x-axis) and test (y-axis) conditions in the double $\log_2$ plots and show $\log_2$-fold expression intensities of lesser than 10. Continuous lines mark the 45-degree diagonal lines, with the dashed lines (right graphs) indicating offsets of 2 or -2.


https://doi.org/10.1016/j.heliyon.2019.e01126

https://doi.org/10.1016/j.heliyon.2019.e01126





**Table 2. Gene ontology analysis on groups of identified genes.** Gene ontology (GO) analyses (functional annotation chart; David Bioinformatics Resources 6.8) were carried out on the highly regulated groups of identified genes shown in the X-Y plots of Fig. 4 (red and green circles). The columns show (from left to right): section (S) of the table, type of contrast, number of identified genes (or unknown genes), annotation (A) source (G, GOTERM_BP_Direct; I: Interpro; U, UP_Keywords), term (Greek characters indicate specific gene groups; *see* Results), number (count) of genes assigned to this term, the percentage share of these genes, Fisher Exact P-value (EASE Score), Q-value (Benjamini). Background colors indicate the respective group of genes. Only dominant terms are shown.

| S | Contrast | Genes | A | Term | Count | % | P | Q |
|---|---|---|---|---|---|---|---|---|
| ***E. coli* HT115 vs. *E. coli* OP50** | | | | | | | | |
| a | Fig. 4a, red circles (up. on *E. coli* OP50) | 137 | U | Ribosomal protein$^{\alpha}$ | 77 | 56.2 | 7.7E-133 | 6.3E-131 |
| | | | U | Ribonucleoprotein$^{\alpha}$ | 77 | 56.2 | 1.0E-124 | 4.0E-123 |
| | | | G | GO:0006412: Translation$^{\alpha}$ | 77 | 56.2 | 1.7E-96 | 3.1E-94 |
| | | | G | GO:0000003: Reproduction | 96 | 70.1 | 7.6E-44 | 6.7E-42 |
| | | | G | GO:0002119: Nematode larval development | 90 | 65.7 | 5.9E-41 | 3.5E-39 |
| | | | G | GO:0009792: Embryo development ending in birth or egg hatching | 101 | 73.7 | 6.2E-36 | 2.7E-34 |
| | | | G | GO:0006915: Apoptotic process | 49 | 35.8 | 1.8E-34 | 6.2E-33 |
| | | | G | GO:0002181: Cytoplasmic translation$^{\alpha}$ | 14 | 10.2 | 2.5E-21 | 7.5E-20 |
| | | | G | GO:0018996: Molting cycle, collagen and cuticulin-based cuticle | 23 | 16.8 | 8.2E-13 | 2.1E-11 |
| | | | U | RNA-binding | 14 | 10.2 | 6.5E-11 | 1.8E-09 |
| | | | G | GO:0008340: Determination of adult lifespan | 36 | 26.3 | 9.3E-11 | 2.0E-09 |
| | | | U | Collagen | 14 | 10.2 | 1.7E-10 | 3.5E-09 |
| b | Fig. 4b, green circles (up. on *E. coli* HT115) | 1013 | I | IPR016187: C-type lectin fold$^{\beta}$ | 53 | 5.2 | 9.9E-21 | 3.4E-18 |
| | | | I | IPR016186: C-type lectin-like$^{\beta}$ | 47 | 4.6 | 1.0E-17 | 1.8E-15 |
| | | | I | IPR001304: C-type lectin$^{\beta}$ | 36 | 3.6 | 1.5E-11 | 1.7E-09 |
| | | | I | IPR003326: TRA-1 regulated | 14 | 1.4 | 4.2E-09 | 3.5E-07 |
| | | | I | IPR006583:PAN-3 domain | 15 | 1.5 | 1.5E-07 | 1.0E-05 |
| | . | | G | GO:0007606: Sensory perception of | 22 | 2.2 | 6.5E-08 | 1.4E-05 |

(*continued on next page*)

**Table 2.** (*Continued*)

| S | Contrast | Genes | A | Term | Count | % | P | Q |
|---|---|---|---|---|---|---|---|---|
| | | | | chemical stimulus[γ] | | | | |
| | | | G | GO:0050907: Detection of chemical stimulus involved in sensory perception[γ] | 27 | 2.7 | 1.4E-06 | 1.5E-04 |
| | | | G | GO:0050911: Detection of chemical stimulus involved in sensory perception of smell[γ] | 22 | 2.2 | 1.1E-05 | 5.9E-04 |
| | | | G | GO:0042048: Olfactory behavior[γ] | 23 | 2.3 | 9.5E-06 | 6.7E-04 |
| | | | G | GO:0043401: Steroid hormone mediated signaling pathway | 22 | 2.2 | 3.6E-05 | 1.5E-03 |
| | | | G | GO:0007186: G-protein coupled receptor signaling pathway[γ] | 38 | 3.8 | 5.6E-05 | 2.0E-03 |
| | | | I | IPR019422: 7TM GPCR, serpentine receptor class h (Srh)[γ] | 23 | 2.3 | 5.0E-04 | 2.8E-02 |
| | | | I | IPR000536: Nuclear hormone receptor, ligand-binding, core | 22 | 2.2 | 7.0E-03 | 2.9E-01 |
| c | Fig. 4b, red circles (up. on *E. coli* OP50) | 500 (3) | G | GO:0042048: Olfactory behavior[γ] | 18 | 3.6 | 6.4E-07 | 7.7E-05 |
| | | | G | GO:0050911: Detection of chemical stimulus involved in sensory perception of smell[γ] | 17 | 3.4 | 1.3E-06 | 7.7E-05 |
| | | | I | IPR019428: 7TM GPCR, serpentine receptor class r (Str)[γ] | 19 | 3.8 | 5.7E-07 | 1.1E-04 |
| | | | I | IPR001304: C-type lectin[β] | 16 | 3.2 | 3.6E-05 | 3.4E-03 |
| | | | G | GO:0050907: Detection of chemical stimulus involved in sensory perception[γ] | 16 | 3.2 | 9.2E-05 | 3.7E-03 |
| | | | I | IPR016187: C-type lectin fold[β] | 17 | 3.4 | 8.9E-05 | 5.6E-03 |
| | | | I | IPR016186: C-type lectin-like[β] | 16 | 3.2 | 1.2E-04 | 5.9E-03 |
| | | | G | GO:0007186: G-protein coupled receptor signaling pathway[γ] | 23 | 4.6 | 3.5E-04 | 1.0E-02 |
| | | | G | GO:0043401: Steroid hormone mediated signaling pathway | 12 | 2.4 | 2.8E-03 | 6.5E-02 |

(*continued on next page*)



https://doi.org/10.1016/j.heliyon.2019.e01126

**Table 2.** (*Continued*)

| S | Contrast | Genes | A | Term | Count | % | P | Q |
|---|---|---|---|---|---|---|---|---|
| ***E. coli* HT115 with 0.1 μmol/L of $CdCl_2$ vs. *E. coli* HT115** | | | | | | | | |
| d | Fig. 4c, red circles (up. on *E. coli* HT115) | 141 (4) | U | Ribosomal protein$^{\alpha}$ | 73 | 51.8 | 1.2E-121 | 8.3E-120 |
| | | | U | Ribonucleoprotein$^{\alpha}$ | 73 | 51.8 | 4.1E-114 | 1.4E-112 |
| | | | G | GO:0006412: Translation$^{\alpha}$ | 70 | 49.6 | 4.5E-87 | 6.1E-85 |
| | | | G | GO:0002119: Nematode larval development | 82 | 58.2 | 2.9E-37 | 2.0E-35 |
| | | | G | GO:0000003: Reproduction | 82 | 58.2 | 5.9E-34 | 2.7E-32 |
| | | | G | GO:0006915: Apoptotic process | 42 | 29.8 | 3.0E-28 | 1.0E-26 |
| | | | G | GO:0009792: Embryo development ending in birth or egg hatching | 86 | 61.0 | 8.4E-27 | 2.3E-25 |
| | | | G | GO:0002181: Cytoplasmic translation$^{\alpha}$ | 14 | 9.9 | 7.3E-22 | 1.6E-20 |
| | | | G | GO:0018996: Molting cycle, collagen and cuticulin-based cuticle | 23 | 16.3 | 1.1E-13 | 2.2E-12 |
| | | | U | Collagen | 15 | 10.6 | 1.8E-11 | 4.1E-10 |
| | | | I | IPR002486: Nematode cuticle collagen, N-terminal | 15 | 10.6 | 1.7E-10 | 4.5E-08 |
| | | | G | GO:0008340: Determination of adult lifespan | 25 | 17.7 | 2.0E-05 | 3.5E-04 |
| e | Fig. 4d, green circles (up. at 0.1 μmol of $CdCl_2$) | 255 | I | IPR027401: Myosin-like IQ motif-containing domain | 3 | 1.2 | 2.8E-03 | 1.7E-01 |
| | | | I | IPR019420: 7TM GPCR, serpentine receptor class bc (Srbc)$^{\gamma}$ | 6 | 2.4 | 1.1E-03 | 1.9E-01 |
| | | | I | IPR004009: Myosin, N-terminal, SH3-like | 3 | 1.2 | 2.2E-03 | 1.9E-01 |
| | | | I | IPR002928: Myosin tail | 3 | 1.2 | 2.2E-03 | 1.9E-01 |
| | | | G | GO:0050907: Detection of chemical stimulus involved in sensory perception$^{\gamma}$ | 9 | 3.5 | 2.1E-03 | 2.2E-01 |
| | | | I | IPR003598: Immunoglobulin subtype 2 | 4 | 1.6 | 1.1E-02 | 3.0E-01 |

(*continued on next page*)



https://doi.org/10.1016/j.heliyon.2019.e01126

**Table 2.** (*Continued*)

| S | Contrast | Genes | A | Term | Count | % | P | Q |
|---|---|---|---|---|---|---|---|---|
| f | Fig. 4d, red circles (up. on *E. coli* HT115) | 2136 (2) | I | IPR000535: Major sperm protein | 79 | 3.7 | 1.2E-57 | 5.8E-55 |
| | | | I | IPR008962: PapD-like | 79 | 3.7 | 1.0E-56 | 2.4E-54 |
| | | | I | IPR002486: Nematode cuticle collagen, N-terminal | 85 | 4.0 | 2.8E-47 | 4.5E-45 |
| | | | I | IPR008160: Collagen triple helix repeat | 67 | 3.1 | 8.6E-34 | 1.0E-31 |
| | | | G | GO:0008360: Regulation of cell shape | 41 | 1.9 | 7.9E-23 | 2.5E-20 |
| | | | G | GO:0006470: Protein dephosphorylation$^{\delta}$ | 54 | 2.5 | 1.6E-22 | 2.5E-20 |
| | | | G | GO:0018105: Peptidyl-serine phosphorylation$^{\delta}$ | 41 | 1.9 | 1.5E-17 | 1.7E-15 |
| | | | G | GO:0035335: Peptidyl-tyrosine dephosphorylation$^{\delta}$ | 34 | 1.6 | 2.9E-14 | 2.3E-12 |
| | | | I | IPR000242: Protein-tyrosine phosphatase, receptor/non-receptor type$^{\delta}$ | 34 | 1.6 | 2.3E-13 | 2.2E-11 |
| | | | I | IPR003595: Protein-tyrosine phosphatase, catalytic$^{\delta}$ | 32 | 1.5 | 2.1E-11 | 1.4E-09 |
| | | | I | IPR006186: Serine/threonine-specific protein phosphatase/bis (5-nucleosyl)-tetraphosphatase$^{\delta}$ | 22 | 1.0 | 1.9E-11 | 1.5E-09 |
| | | | G | GO:0006468: Protein phosphorylation$^{\delta}$ | 62 | 2.9 | 3.4E-09 | 2.2E-07 |
| ***E. coli* HT115 with 10 $\mu$mol/L of $CdCl_2$ vs. *E. coli* HT115** | | | | | | | | |
| g | Fig. 4e, red circles (up. on *E. coli* HT115) | 117 (5) | U | Ribosomal protein$^{\alpha}$ | 58 | 49.6 | 2.2E-93 | 1.5E-91 |
| | | | U | Ribonucleoprotein$^{\alpha}$ | 58 | 49.6 | 7.9E-88 | 2.8E-86 |
| | | | G | GO:0006412: Translation$^{\alpha}$ | 56 | 47.9 | 1.5E-68 | 2.1E-66 |
| | | | G | GO:0002119: Nematode larval development | 67 | 57.3 | 5.6E-31 | 3.9E-29 |
| | | | G | GO:0000003: Reproduction | 68 | 58.1 | 2.4E-29 | 1.1E-27 |
| | | | G | GO:0006915: Apoptotic process | 32 | 27.4 | 1.2E-20 | 4.2E-19 |

(*continued on next page*)





https://doi.org/10.1016/j.heliyon.2019.e01126

**Table 2.** (*Continued*)

| S | Contrast | Genes | A | Term | Count | % | P | Q |
|---|---|---|---|---|---|---|---|---|
| | | | G | GO:0009792: Embryo development ending in birth or egg hatching | 68 | 58.1 | 1.8E-20 | 5.2E-19 |
| | | | G | GO:0018996: Molting cycle, collagen and cuticulin-based cuticle | 17 | 14.5 | 1.4E-09 | 3.3E-08 |
| | | | G | GO:0008340: Determination of adult lifespan | 22 | 18.8 | 1.8E-05 | 3.7E-04 |
| h | Fig. 4f, green circles (up. at 10 $\mu$mol of $CdCl_2$) | 477 | I | IPR016187: C-type lectin fold$^{\beta}$ | 26 | 5.5 | 1.4E-10 | 3.7E-08 |
| | | | I | IPR016186: C-type lectin-like$^{\beta}$ | 24 | 5.0 | 8.7E-10 | 1.1E-07 |
| | | | I | IPR001304: C-type lectin$^{\beta}$ | 22 | 4.6 | 3.5E-09 | 3.0E-07 |
| | | | I | IPR006149: EB domain | 8 | 1.7 | 4.2E-08 | 2.7E-06 |
| | | | G | GO:0030968: Endoplasmic reticulum unfolded protein response$^{\varepsilon}$ | 11 | 2.3 | 3.8E-07 | 6.0E-05 |
| | | | I | IPR003582: Metridin-like ShK toxin$^{\beta}$ | 12 | 2.5 | 1.4E-05 | 7.2E-04 |
| | | | I | IPR003366: CUB-like domain$^{\beta}$ | 8 | 1.7 | 4.9E-05 | 2.1E-03 |
| | | | G | GO:0006952: Defense response$^{\varepsilon}$ | 11 | 2.3 | 3.8E-05 | 3.0E-03 |
| | | | I | IPR002347: Glucose/ribitol dehydrogenase | 9 | 1.9 | 1.7E-04 | 6.0E-03 |
| | | | G | GO:0045087: Innate immune response$^{\beta}$ | 16 | 3.4 | 3.2E-04 | 1.7E-02 |
| i | Fig. 4f, red circles (up. on *E. coli* HT115) | 724 (1) | G | GO:0007186: G-protein coupled receptor signaling pathway$^{\gamma}$ | 45 | 6.2 | 3.9E-14 | 5.1E-12 |
| | | | G | GO:0050911: Detection of chemical stimulus involved in sensory perception of smell$^{\gamma}$ | 25 | 3.5 | 3.8E-11 | 1.7E-09 |
| | | | G | GO:0050907: Detection of chemical stimulus involved in sensory perception$^{\gamma}$ | 28 | 3.9 | 3.0E-11 | 2.0E-09 |
| | | | G | GO:0042048: Olfactory behavior$^{\gamma}$ | 25 | 3.5 | 1.5E-10 | 5.0E-09 |
| | | | G | GO:0007606: Sensory perception of chemical stimulus$^{\gamma}$ | 18 | 2.5 | 5.0E-08 | 1.3E-06 |

(*continued on next page*)



https://doi.org/10.1016/j.heliyon.2019.e01126

**Table 2.** (*Continued*)

| S | Contrast | Genes | A | Term | Count | % | P | Q |
|---|---|---|---|---|---|---|---|---|
| | | | I | IPR019428: 7TM GPCR, serpentine receptor class r (Str)[γ] | 23 | 3.2 | 5.3E-07 | 1.2E-04 |
| | | | I | IPR019422: 7TM GPCR, serpentine receptor class h (Srh)[γ] | 20 | 2.8 | 5.2E-05 | 6.2E-03 |
| | | | I | IPR012885: F-box associated domain, type 2 | 15 | 2.1 | 4.3E-04 | 3.4E-02 |
| | | | I | IPR001810: F-box domain, cyclin-like | 22 | 3.0 | 1.2E-03 | 4.5E-02 |
| | | | I | IPR017452: GPCR, rhodopsin-like, 7TM[γ] | 25 | 3.5 | 1.1E-03 | 4.9E-02 |
| | | | I | IPR019427: 7TM GPCR, serpentine receptor class w (Srw)[γ] | 13 | 1.8 | 1.0E-03 | 6.0E-02 |
| ***E. coli* HT115 with 10 μmol/L of $CdCl_2$ vs. *E. coli* HT115 with 0.1 μmol/L of $CdCl_2$** | | | | | | | | |
| j | Fig. 4g, green circles (up. at 10 μmol of $CdCl_2$) | 129 | U | Ribosomal protein[α] | 71 | 55.0 | 1.2E-120 | 1.3E-117 |
| | | | U | Ribonucleoprotein[α] | 71 | 55.0 | 2.2E-113 | 2.3E-110 |
| | | | G | GO:0006412: Translation[α] | 72 | 55.8 | 1.3E-90 | 1.5E-87 |
| | | | G | GO:0002119: Nematode larval development | 81 | 62.8 | 9.6E-36 | 1.1E-32 |
| | | | G | GO:0000003: Reproduction | 83 | 64.3 | 1.5E-34 | 1.8E-31 |
| | | | G | GO:0006915: Apoptotic process | 45 | 34.9 | 1.4E-31 | 1.6E-28 |
| | | | G | GO:0009792: Embryo development ending in birth or egg hatching | 89 | 69.0 | 3.9E-29 | 4.6E-26 |
| | | | G | GO:0018996: Molting cycle, collagen and cuticulin-based cuticle | 22 | 17.1 | 1.3E-12 | 1.5E-09 |
| | | | U | RNA-binding | 14 | 10.9 | 3.0E-11 | 3.2E-08 |
| | | | U | Collagen | 14 | 10.9 | 8.0E-11 | 8.4E-08 |
| | | | I | IPR002486: Nematode cuticle collagen, N-terminal | 14 | 10.9 | 1.2E-09 | 1.6E-06 |
| | | | G | GO:0008340: Determination of adult lifespan | 29 | 22.5 | 1.9E-07 | 2.2E-04 |

(*continued on next page*)



https://doi.org/10.1016/j.heliyon.2019.e01126

**Table 2.** (*Continued*)

| S | Contrast | Genes | A | Term | Count | % | P | Q |
|---|---|---|---|---|---|---|---|---|
| k | Fig. 4h, green circles (up. at 10 $\mu$mol of $CdCl_2$) | 1799 (1) | I | IPR002486: Nematode cuticle collagen, N-terminal | 83 | 4.6 | 5.6E-49 | 2.5E-46 |
| | | | I | IPR000535: Major sperm protein | 69 | 3.8 | 1.1E-47 | 2.5E-45 |
| | | | I | IPR008962: PapD-like | 69 | 3.8 | 5.7E-47 | 8.6E-45 |
| | | | I | IPR008160: Collagen triple helix repeat | 68 | 3.8 | 6.7E-38 | 7.6E-36 |
| | | | G | GO:0006470: Protein dephosphorylation$^{\delta}$ | 53 | 2.9 | 6.1E-23 | 1.8E-20 |
| | | | G | GO:0008360: Regulation of cell shape | 39 | 2.2 | 9.2E-22 | 1.4E-19 |
| | | | G | GO:0035335: Peptidyl-tyrosine dephosphorylation$^{\delta}$ | 37 | 2.1 | 1.0E-17 | 1.0E-15 |
| | | | I | IPR000242: Protein-tyrosine phosphatase, receptor/non-receptor type$^{\delta}$ | 37 | 2.1 | 1.8E-17 | 1.6E-15 |
| | | | G | GO:0018105: Peptidyl-serine phosphorylation$^{\delta}$ | 39 | 2.2 | 8.5E-17 | 8.4E-15 |
| | | | I | IPR003595: Protein-tyrosine phosphatase, catalytic$^{\delta}$ | 34 | 1.9 | 2.6E-14 | 2.0E-12 |
| | | | I | IPR003582: Metridin-like ShK toxin$^{\beta}$ | 34 | 1.9 | 6.1E-12 | 3.9E-10 |
| | | | G | GO:0045087: Innate immune response$^{\beta}$ | 49 | 2.7 | 2.3E-09 | 1.4E-07 |
| | | | I | IPR001304: C-type lectin$^{\beta}$ | 43 | 2.4 | 3.2E-08 | 1.8E-06 |
| | | | I | IPR016186: C-type lectin-like$^{\beta}$ | 46 | 2.6 | 3.7E-08 | 1.8E-06 |
| | | | I | IPR016187: C-type lectin fold$^{\beta}$ | 48 | 2.7 | 5.8E-08 | 2.6E-06 |
| | | | G | GO:0006468: Protein phosphorylation$^{\delta}$ | 55 | 3.1 | 2.1E-07 | 1.1E-05 |
| | | | I | IPR011009: Protein kinase-like domain$^{\delta}$ | 68 | 3.8 | 5.1E-07 | 2.1E-05 |
| | | | I | IPR000719: Protein kinase, catalytic domain$^{\delta}$ | 58 | 3.2 | 5.2E-06 | 2.0E-04 |
| | | | I | IPR017441: Protein kinase, ATP binding site$^{\delta}$ | 40 | 2.2 | 2.1E-05 | 7.4E-04 |

(*continued on next page*)



https://doi.org/10.1016/j.heliyon.2019.e01126

**Table 2.** (*Continued*)

| S | Contrast | Genes | A | Term | Count | % | P | Q |
|---|---|---|---|---|---|---|---|---|
| l | Fig. 4h, red circles (up. at 0.1 $\mu$mol of $CdCl_2$) | 375 (1) | G | GO:0050907: Detection of chemical stimulus involved in sensory perception$^{\gamma}$ | 14 | 3.7 | 1.9E-05 | 2.0E-03 |
| | | | G | GO:0050911: Detection of chemical stimulus involved in sensory perception of smell$^{\gamma}$ | 12 | 3.2 | 4.8E-05 | 2.5E-03 |
| | | | G | GO:0042048: Olfactory behavior$^{\gamma}$ | 12 | 3.2 | 8.7E-05 | 3.1E-03 |
| | | | G | GO:0007186: G-protein coupled receptor signaling pathway$^{\gamma}$ | 19 | 5.1 | 9.8E-05 | 2.6E-03 |
| | | | I | IPR019428: 7TM GPCR, serpentine receptor class r (Str)$^{\gamma}$ | 12 | 3.2 | 3.7E-04 | 7.0E-02 |
| | | | I | IPR001436: Alpha crystallin/Heat shock protein$^{\varepsilon}$ | 4 | 1.1 | 1.6E-03 | 1.4E-01 |
| | | | I | IPR002068: Alpha crystallin/Hsp20 domain$^{\varepsilon}$ | 4 | 1.1 | 1.8E-03 | 1.1E-01 |
| | | | I | IPR008978: HSP20-like chaperone$^{\varepsilon}$ | 4 | 1.1 | 3.8E-03 | 1.7E-01 |
| | | | I | IPR019422: 7TM GPCR, serpentine receptor class h (Srh)$^{\gamma}$ | 10 | 2.7 | 6.8E-03 | 2.3E-01 |
| | | | G | GO:0009408: Response to heat$^{\varepsilon}$ | 5 | 1.3 | 6.8E-03 | 1.4E-01 |



https://doi.org/10.1016/j.heliyon.2019.e01126

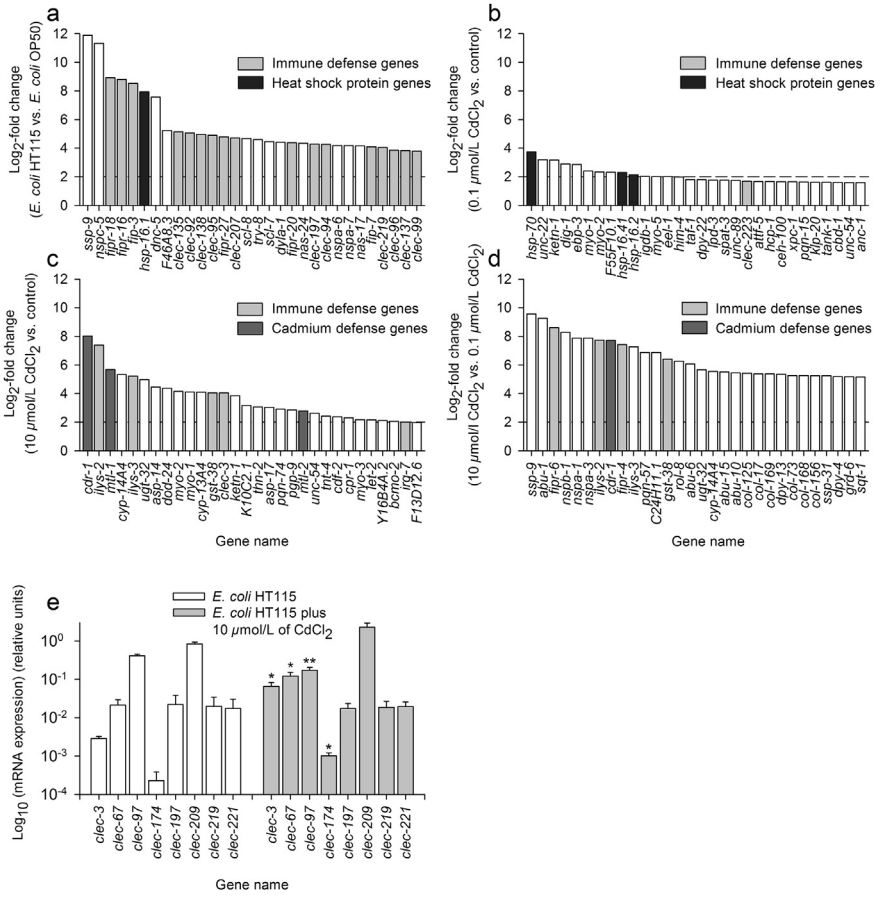


**Fig. 5.** Highly upregulated genes from transcriptome profiling and the mRNA level of different C-type lectins at cadmium exposure. $Log_2$-fold changes in the expression of differentially expressed genes (DEGs), which showed the highest level of upregulation, are shown for the contrasts (a) *E. coli* HT115 vs. *E. coli* OP50 feeding or (b) 0.1 $\mu$mol/L of $CdCl_2$ vs. 0 $\mu$mol/L of $CdCl_2$ (control), (c) 10 $\mu$mol/L of $CdCl_2$ vs. 0 $\mu$mol/L of $CdCl_2$ (control), and (d) 10 $\mu$mol/L of $CdCl_2$ vs. 0.1 $\mu$mol/L of $CdCl_2$, with *E. coli* HT115 as bacterial diet in b−d. Several gene groups are highlighted by different colors: gray bars (immune defense genes), black bars (genes for heat shock proteins), dark-gray bars (cadmium defense genes). The relative quantity of transcripts for different C-type lectins was measured by qRT-PCR (using *cdc-42* as housekeeping gene) in the RNA-Seq samples from synchronized adult wild type worms, which were bred on NGM plates containing 0 or 10 $\mu$mol/L of $CdCl_2$, with *E. coli* HT115 as bacterial diet (mean ± SE; per $CdCl_2$ concentration during breeding, $n = 3$ biological replicates). Asterisks mark significant differences between control and cadmium-treated worms ($^{*}P \leq 0.05$, $^{**}P \leq 0.01$; two-tailed t-test).

especially revealed the following annotation terms (in a partly different sequence): reproduction (GO:0000003), embryo development ending in birth or egg hatching (GO:0009792), receptor-mediated endocytosis (GO:0006898), cellular response to DNA damage stimulus (GO:0006974), and locomotion (GO:0040011) in case of a0-a22 (Supplementary Table S2; terms marked with the Greek character α) and translation (GO:0006412), nematode larval development (GO:0002119), reproduction (GO:0000003), and embryo development ending in birth or egg hatching


https://doi.org/10.1016/j.heliyon.2019.e01126

(GO:0009792) in case of b0, b1, and b11 (Supplementary Table S2; terms marked with the Greek character β). However, the DEGs collected in these five or four terms encode quite different proteins, with helicases, protein kinases, or proteins containing armadillo or WD40 repeats dominating in all mentioned terms from a0-a22, and ribosomal proteins, translational and proteasomal proteins dominating in all mentioned terms from b0, b1, and b11. The highest mean expression intensities (centered and normalized $\log_2$-transformed values; *see* Material and methods) were found under the experimental condition 0.1 Cd (or HT115_1) in case of a0-a22 or OP50 in case of b0, b1, and b11 (boldfaced abbreviated worm treatments). GO analyses on the DEG clusters b2 and b21 especially revealed the annotation terms flavonoid biosynthetic process (GO:0009813) and innate immune response (GO:0045087) (Supplementary Table S2; terms marked with the Greek character γ), with the DEGs collected in these terms primarily encoding either UDP-glucuronosyl/UDP-glucosyltransferases or proteins with CUB-like or Gcn5-related N-acetyltransferase (GNAT) domains. The highest mean expression intensities were found under the experimental condition HT115_2 or 10 Cd in case of b2 or b21. Thus, *E. coli* OP50 feeding caused maximal mean expression intensities in the DEG clusters b0, b1, or b11, which primarily encode translational proteins. *E. coli* HT115 feeding or *E. coli* HT115 feeding with 10 μmol/L of $CdCl_2$ in the NGM caused maximal mean expression intensities in the DEG clusters b2 or b21, which frequently encode proteins for flavonoid biosynthetic processes or the innate immune response.

## 4. Discussion

### 4.1. Weak stress

This study aimed to address the effects of weak abiotic and/or biotic stress on the survival probability, stress resistance and gene expression of *C. elegans*. To specify a weak abiotic stress or stressor, the developmental speed of wild type (WT) was determined at different cadmium concentrations (Fig. 1a–j), which revealed a developmental delay already at 50 μmol/L of $CdCl_2$. Accordingly, we reduced the $CdCl_2$ concentration in the nematode growth medium (NGM), on which worms were cultivated, to levels below one hundredth of this concentration (i.e., 0.1 μmol/L of $CdCl_2$), which was hereinafter classified as weak abiotic stress. This choice also considered a previous report, which has shown positive effects of $CdCl_2$ concentrations between 0.01 and 1 μmol/L on enzyme activities [4]. In addition, several experiments were carried out at a significantly higher cadmium concentration in the NGM (10 μmol/L of $CdCl_2$).

To specify a weak biotic stress/stressor, we tested the effects of two different *E. coli* strains as bacterial diet. Short-term survival assays showed a tendentiously but not


https://doi.org/10.1016/j.heliyon.2019.e01126

significantly higher survival probability of adult WT worms on *E. coli* OP50 (Fig. 1k) but a significantly higher survival probability of adult *daf-2*Δ worms on *E. coli* OP50 than on *E. coli* HT115 (Fig. 1n). Long-term survival assays showed no differences in maximum life span but a higher age-specific survival probability of adult WT worms on *E. coli* OP50 than on *E. coli* HT115 (Fig. 2a). Thus, we hereinafter classified *E. coli* HT115 as weak biotic stressor, either as bacterial diet of lower quality or as mildly pathogenic bacterium.

## 4.2. Severe stress

From the data on developmental speed at different cadmium concentrations (Fig. 1a–j), it is evident that a $CdCl_2$ concentration of 10 mmol/L represents a severe abiotic stress/stressor (see also [24]). With regard to a severe biotic stress/stressor, preliminary experiments in our laboratory suggested to use *C. arvensicola* as animal feed. Accordingly, we tested *C. arvensicola* as bacterial diet and found significant negative effects on the short-term survival probability of adult worms of all tested *C. elegans* strains (Fig. 1k–o). Therefore, *C. arvensicola* can be considered as a severe biotic stressor for *C. elegans*. The negative effect of *C. arvensicola* feeding on survival probability was much lower in the stress-tolerant mutant *daf-2*Δ (Fig. 1n) but only slightly higher in the stress-sensitive mutant *daf-16*Δ (Fig. 1o) than in WT. Blocked DAF-2 signaling protected *daf-2*Δ against *C. arvensicola* by a stress-independent reinforcement of nuclear translocations of the non-phosphorylated transcription factor DAF-16. Inhibiting effects of *C. arvensicola* on the intact DAF-2 signaling in WT (to trigger DAF-16 nuclear translocations) were obviously not strong because WT and *daf-16*Δ did not much differ in their survival probability.

## 4.3. Effects of weak abiotic and/or biotic stress on the age-specific survival probability of wild type and different mutant strains

Further experimental series served to detect in identically bred adult worms the effects of different experimental conditions (weak abiotic and/or biotic stress) on their further age-specific survival. In accordance with previous results [43, 44], the signaling mutants *kgb-1*Δ and *pmk-1*Δ (Figs. 1l–m, 2f–j, k–o) showed a lower age-specific survival probability than WT (Figs. 1k, 2a–e) under each tested experimental condition.

Determining the age-specific survival probability of adult WT, *kgb-1*Δ, and *pmk-1*Δ worms showed negative effects of weak biotic stress (Fig. 2a) but positive effects of weak abiotic and biotic stress (Fig. 2c) in WT worms, positive effects of weak biotic stress and weak abiotic and biotic stress (Fig. 2f, i, j) in *kgb-1*Δ worms, and also positive effects of weak biotic stress (Fig. 2k) but negative effects of weak abiotic and biotic stress (Fig. 2m, n, o) in *pmk-1*Δ worms.


https://doi.org/10.1016/j.heliyon.2019.e01126

As the level of fat storage (i.e., available energy resources) is higher in WT on *E. coli* OP50 than on *E. coli* HT115 (e.g., [21]), increases in the age-specific survival probability of WT seem to result from either a bacterial diet causing higher levels of energy resources (*E. coli* OP50) or a combination of weak abiotic and biotic stress (0.1 $\mu$mol/L of $CdCl_2$ and *E. coli* HT115 feeding). Positive effects on the age-specific survival probability of *kgb-1*Δ were achieved by weak biotic stress alone, independent of the absence or presence of weak abiotic stress. The weak biotic stress also promoted the age-specific survival probability of *pmk-1*Δ, however, weak abiotic and biotic stress reduced it severely.

The results in *kgb-1*Δ and *pmk-1*Δ indicate a higher cadmium sensitivity of *pmk-1*Δ. This finding is in accordance with previous results [24], which showed that PMK-1 signaling strongly promotes the cadmium resistance of *C. elegans* mostly via SKN-1 and DAF-16 target gene expression. Thus, while the cadmium resistance of *pmk-1*Δ is impaired by the reduction in DAF-16 target gene expression, there were no specific effects of KGB-1 signaling on DAF-16 activity under hyperosmotic stress [44]. *kgb-1*Δ even showed an improved hyperosmotic tolerance likely due to an elevated level of membrane transporters. Accordingly, the absent negative effects of weak abiotic stress in *kgb-1*Δ may be explained by cadmium exports via membrane transports and/or non-reduced DAF-16 activity. The promotion of the age-specific survival probability of *kgb-1*Δ or *pmk-1*Δ by weak biotic stress indicates that an alternative stress mechanism exists, which is independent of KGB-1 or PMK-1 but upregulated on *E. coli* HT115. Possibly, elevated DAF-16 activity, not induced by PMK-1 signaling but by a signal related to the bacterial diet, constitutes such a mechanism.

Wild type has the immunosuppressive KGB-1 and PMK-1 pathways [25] at its disposal, which also provide resistance to several abiotic stressors [23, 24, 44]. However, the use of these more stress-specific signaling cascades may entail higher energy demands for signal processing (e.g., for the numerous phosphorylation reactions in the MAPK signaling network) than the rather unspecific DAF-2 pathway, where the stress-mediated inhibition of the DAF-2 receptor simply shuts off DAF-2 signaling, which inevitably results in the nuclear translocation of the non-phosphorylated transcription factor DAF-16. This would imply an energetic support for MAPK signaling on a bacterial diet causing higher levels of energy resources (*E. coli* OP50). Fully active cellular processes, including MAPK signaling, on *E. coli* OP50 as bacterial diet may then have promoted the stress resistance and age-specific survival probability of WT. The more stress-sensitive *kgb-1*Δ and *pmk-1*Δ mutants may predominantly use elevated DAF-16 target gene expression, triggered in this case by weak biotic stress (*E. coli* HT115), to achieve a higher stress resistance and age-specific survival probability. The more stress-insensitive WT, however, may need an additional weak abiotic stress, besides the weak biotic stress, as signal to promote DAF-16 activity and reach a higher stress resistance and age-


https://doi.org/10.1016/j.heliyon.2019.e01126

specific survival probability via elevated DAF-16 target gene expression. These assertions are assessed in further detail below.

## 4.4. Effects of weak abiotic and/or biotic stress during breeding on the resistance of wild type to severe stress

The resistance of adult WT worms to severe stress was determined after breeding on *E. coli* OP50 or *E. coli* HT115 with 0, 0.1, or 10 $\mu$mol/L $CdCl_2$ in the NGM (Fig. 3). Especially *E. coli* OP50 feeding but also weak abiotic and biotic stress (0.1 $\mu$mol/L of $CdCl_2$ and *E. coli* HT115 feeding) during breeding improved the resistance to severe abiotic stress (10 mmol/L $CdCl_2$). Especially weak abiotic and biotic stress but also weak abiotic stress (0.1 $\mu$mol/L of $CdCl_2$, with *E. coli* OP50 as bacterial diet) or weak biotic stress (*E. coli* HT115 feeding) during breeding improved the resistance to severe biotic stress (*C. arvensicola* feeding). Thus, a bacterial diet causing higher levels of energy resources or weak abiotic and biotic stress during breeding promoted the resistance to severe abiotic stress, whereas weak abiotic and biotic stress or weak abiotic/biotic stress alone during breeding promoted the resistance to severe biotic stress.

These experiments and the transcriptomic experiments (see below) served to detect the effects of different experimental conditions (weak abiotic and/or biotic stress) during breeding on stress resistance and gene regulation in adult WT. Other survival experiments (Fig. 2), however, were carried out on identically bred adult worms to study the effects of different experimental conditions on their age-specific survival probability. Thus, the results from the former (stress resistance, gene regulation) and the latter (age-specific survival probability) experiments have to be compared with some care, although the results of both types of survival experiments were quite similar (higher stress resistance and age-specific survival probability on *E. coli* OP50 or *E. coli* HT115 with 0.1 $\mu$mol/L of $CdCl_2$ in the NGM).

## 4.5. Effects of weak abiotic and/or biotic stress during breeding on gene expression in wild type

To explore the genetic basis of these results, transcriptome profiling by RNA-Seq was performed. In this context, however, it must be remembered that mRNA levels and the abundance or, moreover, the activity of proteins are not necessarily linked to each other. Although changes in mRNA level mostly take place with the aim of inducing changes in protein level, which frequently take place on different time scales [45, 46], at least repressed mRNA expression may not result in changes of the corresponding protein profile [47]. The reason is that a multitude of processes downstream of mRNA synthesis (e.g., posttranscriptional regulation, protein degradation, protein phosphorylation) may affect the total amount and/or activity of proteins in the cell [48]. Stressor-induced impairments of translational efficiency or protein structure may also require an upregulation of mRNA expression to maintain


https://doi.org/10.1016/j.heliyon.2019.e01126

the protein level. Accordingly, the performed RNA-Seq study can be assessed as starting point for further mechanistic studies.

RNA-Seq was carried out on synchronized adult WT worms, which were bred on *E. coli* OP50 or *E. coli* HT115 (Figs. 4a–b and S1a–b) or on *E. coli* HT115, with 0, 0.1, or 10 $\mu$mol/L of $CdCl_2$ in the NGM (Figs. 4c–h and S1c-h). The transcriptomic data were functionally analyzed by GO analyses with regard to up- or downregulated genes (Fig. 4, Table 2) or DEGs (Fig. S1, Table S1) between two different experimental conditions.

The regulated gene or DEG groups with higher expression intensity (Figs. 4 and S1, left graphs) were consistently related to terms referring to protein biosynthesis (Tables 2 and S1). The expression intensity of these genes or DEGs was higher on *E. coli* OP50 than on *E. coli* HT115, higher on *E. coli* HT115 than on *E. coli* HT115 with 0.1 or 10 $\mu$mol/L of $CdCl_2$ in the NGM, and higher on *E. coli* HT115 with 10 than with 0.1 $\mu$mol/L of $CdCl_2$ in the NGM. Cluster and GO analyses (Fig. S2 and Table S2) also showed maxima in mean expression intensity on *E. coli* OP50 and minima on *E. coli* HT115 with 0.1 $\mu$mol/L of $CdCl_2$ in the NGM in the DEG clusters b0, b1, and b11, which primarily encode translational proteins. Consequently, gene expression for protein biosynthesis decreased in the order: *E. coli* OP50 feeding, *E. coli* HT115 feeding, *E. coli* HT115 feeding with 10 $\mu$mol/L of $CdCl_2$, and *E. coli* HT115 feeding with 0.1 $\mu$mol/L of $CdCl_2$ in the NGM. The higher level of gene expression for translational processes on *E. coli* OP50 indicates a higher level of available energy resources, as an increase in the number of translational machineries involves higher energy (ATP) demands during their operation [49]. The lower level of gene expression for protein biosynthesis under the other experimental conditions may be related to the negative effects of DAF-16 on the expression of an essential component (DAF-15/Raptor) of target of rapamycin (TOR) signaling, which promotes protein biosynthesis and growth [50]. Nuclear DAF-16 inhibits *daf-15* expression [51], which inhibits in the long-term TOR signaling, with negative effects on gene expression for protein biosynthesis. This interpretation suggests that negative effects of nuclear DAF-16 on TOR signaling and protein biosynthesis during breeding may have increased in the sequence *E. coli* OP50 feeding, *E. coli* HT115 feeding, *E. coli* HT115 feeding with 10 $\mu$mol/L of $CdCl_2$, and *E. coli* HT115 feeding with 0.1 $\mu$mol/L of $CdCl_2$ in the NGM. As this interpretation matches several of the former results on WT and their interpretation (see above), we checked for further indications of lower nuclear DAF-16 levels on *E. coli* OP50 and higher nuclear DAF-16 levels and positively controlled DAF-16 target gene expression under the other experimental conditions (see below).

The regulated gene or DEG groups with lower expression intensity (Figs. 4 and S1, right graphs) were consistently related to terms referring to innate immune response, sensory perception, signaling, or stress response (Tables 2 and S1).


https://doi.org/10.1016/j.heliyon.2019.e01126

Genes or DEGs likely involved in the innate immune response or in signaling processes were primarily upregulated on *E. coli* HT115 and *E. coli* HT115 with 10 $\mu$mol/L of $CdCl_2$ (*see* Results). The most intensely upregulated DEGs frequently encoded putative immune defense genes on *E. coli* HT115 (Fig. 5a). Several of these DEGs were also found on *E. coli* HT115 with 10 $\mu$mol of $CdCl_2$ (Fig. 5c, d), and there was a significant upregulation of several C-type lectin mRNAs in worms exposed to 10 $\mu$mol/L of $CdCl_2$ in comparison to control worms, with *E. coli* HT115 feeding in both cases (Fig. 5e). The immune response genes encoded C-type lectins, proteins carrying a CUB-like domain structure, fungus-induced proteins, and metridin-like ShK toxins. Most of them are target genes of PMK-1 signaling [26]. Thus, it is possible that the parallel upregulation of genes or DEGs for signaling and innate immune responses is due to an upregulated gene expression for PMK-1 signaling and associated immune responses.

Genes or DEGs likely involved in sensory perception were never upregulated on *E. coli* HT115 with 10 $\mu$mol/L of $CdCl_2$ (*see* Results), which may be related to toxic effects, as a loss of neurons was shown in *C. elegans* already at relatively low cadmium concentrations (2.5 $\mu$mol/L of $CdCl_2$ [52]). G-protein-coupled receptors (GPCR) can be activated by chemical stimuli and of 100 candidate chemoreceptors, 60 were found to be expressed in chemosensory neurons in *C. elegans* [53]. These receptors are predicted to contain seven transmembrane receptor domains (7-TM), including the serpentine receptors for which responses to odorants were suggested [54, 55]. As zebrafish larvae showed significantly lower behavioral responses to L-cysteine stimuli under external cadmium concentrations between 1 and 5 $\mu$mol/L of $CdCl_2$ [56], the ability of *C. elegans* to respond to chemical signals and detect feed may also be impaired at higher cadmium concentrations.

Genes or DEGs likely involved in stress responses were never upregulated on *E. coli* OP50 (*see* Results). The most intensely upregulated DEGs encoded several heat shock proteins (HSP-70; small heat shock proteins/α-crystallins) (Fig. 5b) or cadmium defense proteins (CDR-1, MTL-1/2) (Fig. 5c, d) on *E. coli* HT115, with 0.1 or 10 $\mu$mol of $CdCl_2$ in the NGM. The gene *cdr-1* is an SKN-1 target gene controlled by PMK-1 signaling [57]. Other genes are DAF-16 target genes [37, 58], such as genes for small heat shock proteins (HSP-16.2/16.41) or metallothioneins (MTL-1/2). The terms referring to insulin-like signaling, which were found on *E. coli* HT115 (Table S1: h), included up to thirteen signaling substances (insulin-like peptides) for the DAF-2 receptor. As there are activating or inhibiting insulin-like peptides [59], it is not clear whether they can activate or inhibit DAF-2 signaling. But it is evident that *E. coli* HT115 feeding caused a greater involvement of DAF-2 signaling. The term 'response to heat', which was found on *E. coli* HT115 with 0.1 $\mu$mol of $CdCl_2$ (Table 2: l; Table S1: e) included up to five genes associated with DAF-2 signaling and DAF-16 target gene expression (e.g., *hsp-16.2/16.41*). Thus, DAF-2 signaling and DAF-16 target gene expression seem to be involved


https://doi.org/10.1016/j.heliyon.2019.e01126

in the stress responses of WT on *E. coli* HT115, especially when 0.1 or 10 $\mu$mol of $CdCl_2$ was added to the NGM.

These results support the former suggestion (see above) of lower nuclear DAF-16 levels on *E. coli* OP50 and higher nuclear DAF-16 levels on *E. coli* HT115 with 0.1 or 10 $\mu$mol/L of $CdCl_2$. As PMK-1 signaling is possibly involved in the stress and immune responses on *E. coli* HT115 and *E. coli* HT115 with 10 $\mu$mol/L of $CdCl_2$ (see above), DAF-16 target expression may be particularly important on *E. coli* HT115 with 0.1 $\mu$mol/L of $CdCl_2$.

## 4.6. Conclusion

PMK-1 signaling and DAF-16 target gene expression provide resistance to abiotic and/or biotic stress. On a bacterial diet causing higher levels of energy resources (*E. coli* OP50), the available energy appeared to be sufficient to operate MAPK signaling, protein biosynthesis, and other cellular processes at simultaneously high levels, which probably caused the high resistance to severe abiotic stress (10 mmol/L of $CdCl_2$) and promoted the age-specific survival probability of WT.

In case, energy resources were more restricted (*E. coli* HT115 feeding), gene expression for protein biosynthesis was downregulated but PMK-1 signaling and the associated expression of immune response genes were obviously upregulated, triggered by a signal related to this bacterial diet. The upregulation of PMK-1 signaling under weak biotic stress likely caused the higher resistance of adult WT to severe biotic stress (*C. arvensicola* feeding). Additionally, there were first indications for a greater involvement of DAF-2 signaling under this experimental condition.

An additional weak abiotic stress (0.1 $\mu$mol/L of $CdCl_2$), besides the weak biotic stress (*E. coli* HT115), led to a further reduction in gene expression for protein biosynthesis. The upregulation of DAF-16 target gene expression (e.g., genes for small heat shock proteins) under this stress condition probably caused the high (higher) resistance of adult WT to severe biotic (abiotic) stress and was possibly responsible for the higher age-specific survival probability of adult WT under this experimental condition.

A clearly higher additional abiotic stress (10 $\mu$mol/L of $CdCl_2$), however, re-intensified in a certain degree gene expression for protein biosynthesis as well as PMK-1 signaling and associated gene expression (*cdr-1*, immune response genes). DAF-16 target gene expression was still operating, with a focus now on the expression of cadmium defense genes (e.g., genes for metallothioneins). This and a possibly lower DAF-16 activity under this stress condition than under weak abiotic stress (0.1 $\mu$mol/L of $CdCl_2$) may have been responsible for the missing preparation of WT for the subsequent exposure to severe biotic stress.


https://doi.org/10.1016/j.heliyon.2019.e01126

Thus, feeding with bacteria providing higher energy resources or weak abiotic and biotic stress during breeding particularly positively affect the stress resistance of adult worms, which is likely based on non-restricted MAPK signaling and protein biosynthesis or DAF-16 target gene expression. Adult worms also benefit from these environmental conditions as they improve their age-specific survival probability.

## Declarations

### Author contribution statement

Ramona Dölling: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Martha N. Mendelski: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Rüdiger J. Paul: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

### Funding statement

This work was supported by the Deutsche Forschungsgemeinschaft (Pa 308/13-1).

### Competing interest statement

The authors declare no conflict of interest.

### Additional information

Data associated with this study has been deposited at the NCBI Gene Expression Omnibus (GEO) under the accession number GSE123183.

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2019.e01126.

## Acknowledgements

We thank Jörg Hartmann and Ulrike Gigengack (Institute of Zoophysiology, WWU Münster) for supporting measurements and data sets.

## References

[1] E.J. Calabrese, L.A. Baldwin, Defining hormesis, Hum. Exp. Toxicol. 21 (2002) 91–97.

[2] A.R.D. Stebbing, Hormesis-stimulation of colony growth in *Campanularia flexuosa* (hydrozoa) by copper, cadmium and other toxicants, Aquat. Toxicol. 1 (1981) 227−238.

[3] N. Saul, K. Pietsch, R. Menzel, S.R. Stürzenbaum, C.E.W. Steinberg, The longevity effect of tannic acid in *Caenorhabditis elegans*: disposable soma meets Hormesis, J. Gerontol. A Biol. Sci. Med. Sci. 65 (6) (2010) 626−635.

[4] U.N. Rai, Meetu Gupta, R.D. Tripathi, P. Chandra, Cadmium regulated nitrate reductase activity in *Hydrilla verticillata* (1.F.) royale, Water Air Soil Pollut. 106 (1998) 171−177.

[5] G. Bertin, D. Averbeck, Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review), Biochimie 88 (2006) 1549−1559.

[6] E.J. Martinez-Finley, M. Aschner, Revelations from the nematode *Caenorhabditis elegans* on the complex interplay of metal toxicological mechanisms, J. Toxicol. 2011 (2011) 895236.

[7] A. Broeks, B. Gerrard, R. Allikmets, M. Dean, R.H. Plasterk, Homologues of the human multidrug resistance genes MRP and MDR contribute to heavy metal resistance in the soil nematode *Caenorhabditis elegans*, EMBO J. 15 (1996) 6132−6143.

[8] J.Y. Roh, L. Junho, C. Jinhee, Assessment of stress-related gene expression in the heavy metal-exposed nematode *Caenorhabditis elegans*: a potential biomarker for metal-induced toxicity monitoring and envriomental risk assesment, Environ. Toxicol. Chem. 25 (11) (2006) 2946−2956.

[9] L. Hughes, J.G. Bundy, E.J. Want, P. Kille, S.R. Stürzenbaum, The metabolomic responses of *Caenorhabditis elegans* to cadmium are largely independent of metallothionein status, but dominated by changes in cystathionin and phytochelatin, J. Proteome Res. 8 (7) (2009) 3512−3519.

[10] M. Leroy, T. Mosser, X. Manière, D. Fernández Alvarez, I. Matic, Pathogen-induced *Caenorhabditis elegans* developmental plasticity has a hormetic effect on the resistance to biotic and abiotic stresses, BMC Evol. Biol. 22 (2012) 187−197.

[11] T. Stiernagle, Maintenance of *C. elegans*, WormBook, 2006. The C. elegans Research Community, WormBook, http://www.wormbook.org.

[12] D.A. Garsin, C.D. Sifri, E. Mylonakis, X. Qin, K.V. Singh, B.E. Murray, S.B. Calderwood, F.M. Ausubel, A simple model host for identifying


https://doi.org/10.1016/j.heliyon.2019.e01126

Gram-positive virulence factors, Proc. Natl. Acad. Sci. Unit. States Am. 98 (19) (2001) 10892−10897.

[13] D. Gems, D.L. Riddle, Genetic, behavioral and environmental determinates of male longevity in *Caenorhabditis elegans*, Genetics 154 (2000) 1597−1610.

[14] D. Garigan, A.L. Hsu, A.G. Fraser, R.D. Kamath, J. Ahringer, C. Kenyon, Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation, Genetics 161 (2002) 1101−1112.

[15] S. Dasgupta, L. Fernadez, L. Kameyama, T. Inada, A. Pappas, D.L. Court, Genetic uncoupling of the dsRNA-binding and RNA cleavage activities of *Escherichia coli* endoribonuclease RNase III- the effect of dsRNA binding on gene expression, Mol. Microbiol. 28 (3) (1998) 629−640.

[16] R.S. Kamath, M. Martinez-Campos, P. Zipperlen, A.,G. Fraser, J. Ahringer, Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*, Genome Biol. 2 (1) (2000) research 0002.1−0002.10.

[17] P. Prehm, S. Strim, B. Jann, K. Jann, Cell-wall lipopolysaccharide from *Escherichia coli* B, FEBS J. 56 (1975) 41−55.

[18] P. Prehm, G. Schmidt, B. Jann, K. Jann, Cell-wall lipopolysaccharide from *Escherichia coli* K 12, FEBS J. (1976) 171−177.

[19] J. Klena, P. Zhan, O. Schwartz, S. Hull, T. Chen, The core lipopolysaccharide of *Escherichia coli* is a ligand for the dendritic-cell-specific intercellular adhesion molecule nonintegrin CD209 receptor, J. Bacteriol. 187 (5) (2005) 1710−1715.

[20] W. Maier, B. Adilov, M. Regenass, J. Alecedo, A neuromedin U receptor acts with the sensory system to modulate food type-dependent effects on *C. elegans* lifespan, PLoS Biol. 8 (5) (2010), e1000376.

[21] K.K. Brooks, B. Laing, J.L. Watts, The influence of bacterial diet on fat storage in *C. elegans*, PLoS One 4 (10) (2009), e7545.

[22] R. Xiao, L. Chun, E.A. Ronan, D.I. Friedman, J. Liu, X.Z. Shawn Xu, RNAi interrogation of dietary modulation of development, metabolism, behavior, and aging in *C. elegans*, Cell Rep. 11 (7) (2015) 1123−1133.

[23] A. Mertenskötter, A. Keshet, P. Gerke, R.J. Paul, The p38 MAPK PMK-1 shows heat-induced nuclear translocation, supports chaperone expression, and affects the heat tolerance of *Caenorhabditis elegans*, Cell Stress Chaperones 3 (2012) 293−306.


https://doi.org/10.1016/j.heliyon.2019.e01126

[24] A. Keshet, A. Mertenskötter, S.A. Winter, V. Brinkmann, R. Dölling, R.J. Paul, PMK-1 p38 MAPK promotes cadmium stress resistance, the expression of SKN-1/Nrf and DAF-16 target genes, and protein biosynthesis in *Caenorhabditis elegans*, Mol. Gen. Genet. 292 (6) (2017) 1341−1361.

[25] D.L. Huffman, L. Abrami, R. Sasik, J. Corbeil, F.G. van der Groot, R.V. Aroian, Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins, Proc. Natl. Acad. Sci. Unit. States Am. 101 (30) (2004) 10995−11000.

[26] E.R. Troemel, S.W. Chu, V. Reinke, A.S. Lee, F.M. Ausuble, D.H. Kim, p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*, PLoS Genet. (2006), e183.

[27] D.D. Bolz, J.L. Tenor, A. Aballay, A Conserved PMK-1/p38 MAPK is required in *Caenorhabditis elegans* tissue-specific immune response to *Yersinia pestis* infection, J. Biol. Chem. 285 (14) (2010) 10832−10840.

[28] H.L. Cash, C.V. Whitham, L.V. Hooper, Symbiotic bacteria direct expression of an intestinal bactericidal lectin, Science 313 (5790) (2006) 1126−1130.

[29] H. Schulenburg, M.P. Hoeppner, J. Weiner III, E. Bornberg-Bauer, Specificity of the innate immune system and diversity of C-type lectin domain (CTLD) proteins in the nematode *Caenorhbditis elegans*, Immunobiology 213 (2008) 237−250.

[30] O. Castañeda, V. Sotolongo, A.M. Amor, R. Stöcklin, A.J. Anderson, A.L. Harvey, A. Engström, C. Wernstedt, E. Karlsson, Characterization of a potassium channel toxin from the Caribbean sea anemone *Stichodactyla helianthus*, Toxicon 33 (1995) 603−613.

[31] J.H. Thomas, Analysis of homologous gene clusters in *Caenorhabditis elegans* reveals striking regional cluster domains, Genetics 172 (2006) (2006) 127−143.

[32] M. Koga, R. Zwaal, K.L. Guan, L. Avery, Y. Ohshima, A *Caenorhabditis elegans* MAP kinase kinase, MEK-1, is involved in stress response, EMBO J. 19 (19) (2000) 5148−5156.

[33] T. Mizuno, K. Fujiki, A. Sasakawa, N. Hisamoto, K. Matsumoto, Role of the C*aenorhabditis elegans* Shc adaptor protein in the c-Jun N-terminal kinase signaling pathway, Mol. Cell Biol. 28 (23) (2008) 7041−7049.

[34] K. Fujiki, T. Mizuno, N. Hisamoto, K. Matsumoto K, The *Caenorhabditis elegans* Ste20-related kinase and Rac-type small GTPase regulate the c-Jun N-terminal kinase signaling pathway mediating the stress response, Mol. Cell Biol. 30 (2010) 995−1003.


https://doi.org/10.1016/j.heliyon.2019.e01126

[35] A. Hattori, T. Mizuno, M. Akamatsu, N. Hisamoto, K. Matsumoto, The *Caenorhabditis elegans* JNK signaling pathway activates expression of stress response genes by depressing the Fos/HDAC repressor complex, PLoS Genet. 9 (2) (2013), e1003315.

[36] J.A. Young, A. Dillin, MAPping innate immunity, Proc. Natl. Acad. Sci. Unit. States Am. 101 (35) (2004) 12781−12782.

[37] C.T. Murphy, S.A. McCarroll, C.I. Bargmann, A. Fraser, R.S. Kamath, J. Ahringer, H. Li, C. Kenyon, Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*, Nature 424 (2003) 277−283.

[38] C. Kenyon, J. Chang, E. Gensch, A. Rudner, R. Tabtiang, A *C. elegans* mutant lives twice as long as wild type, Letters to Nature 366 (1993) 461−464.

[39] D.L. Miller, M.B. Roth, *C. elegans* are protected from lethal hypoxia by an embryonic diapause, Curr. Biol. 19 (14) (2009) 1233−1237.

[40] S. Tarazona, F. García-Alcade, J. Dopazo, A. Ferrer, A. Conesa, Differential expression in RNA-seq: a matter of depth, Genome Res. 21 (12) (2011) 2213−2223.

[41] J. Haimes, M. Kelley, Demonstration of a ΔΔ C Q Calculation Method to Compute Relative Gene Expression from qPCR Data, Thermo Fisher Scientific, Lafayette, Colo, USA, 2010.

[42] D. Hoogewijs, K. Houthoofd, F. Matthijssens, J. Vandesompele, R.J. Vanfleteren, Selection and validation of a set of reliable Mol Genet Genomics reference genes for quantitative *sod* gene expression analysis in *C. elegans*, BMC Mol. Biol. 9 (9) (2008).

[43] M. Uno, S. Honjoh, M. Matsuda, H. Hoshikawa, S. Kishimoto, T. Yamamoto, M. Ebisuya, T. Yamamoto, K. Matsumoto, E. Nishida, A fasting-responsive signaling pathway that extends life span in *C. elegans*, Cell Rep. 3 (2013) 79−91.

[44] P. Gerke, A. Keshet, A. Mertenskötter, R.J. Paul, The JNK-like MAPK KGB-1 of *Caenorhabditis elegans* promotes reproduction, lifespan, and gene expression for protein biosynthesis and germline homeostasis but interferes with hyperosmotic stress tolerance, Cell. Physiol. Biochem. 34 (6) (2014) 1951−1973.

[45] D. Becker, B.F. Brinkmann, B. Zeis, R.J. Paul, Acute changes in temperature or oxygen availability induce ROS fluctuations in *Daphnia magna* linked with fluctuations of reduced and oxidized glutathione, catalase activity and gene (haemoglobin) expression, Biol. Cell. 103 (8) (2011) 351−363.

 https://doi.org/10.1016/j.heliyon.2019.e01126

[46] E. Klumpen, N. Hoffschröer, B. Zeis, U. Gigengack, E. Dohmen, R.J. Paul, Reactive oxygen species (ROS) and the heat stress response of *Daphnia pulex*: ROS-mediated activation of hypoxia-inducible factor 1 (HIF-1) and heat shock factor 1 (HSF-1) and the clustered expression of stress genes, Biol. Cell. 109 (1) (2017) 39−64.

[47] D.H. Lackner, M.W. Schmidt, W. Shuangding, D.A. Wolf, J. Bähler, Regulation of transcriptome, translation, and proteome in response to environmental stress in fission yeast, Genome Biol. 13 (2012) R25.

[48] M.E. Feder, J.C. Walser, The biological limitations of transcriptomics in elucidating stress and stress responses, J. Evol. Biol. 18 (2005) 901−910.

[49] F. Buttgereit, M.D. Brand, A hierarchy of ATP-consuming processes in mammalian cells, Biochem. J. 312 (1) (1995) 163−167.

[50] S. Wullschleger, R. Loewith, M.N. Hall, TOR signaling in growth and metabolism, Cell 124 (2006) 471−484.

[51] K. Jia, C. Chen, D.L. Riddle, The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span, Development 131 (2004) 3897−3906.

[52] M. Du, D. Wang, The neurotoxic effect of heavy metal exposure on GABAergic nervous system in nematode *Caenorhabditis elegans*, Environ. Toxicol. Pharmacol. 27 (2009), 314−310.

[53] C.I. Bargmann, Chemosensation in *C. elegans*, 2006. http://www.wormbook.org.

[54] H.R. Bourne, How receptors talk to trimeric G-proteins, Curr. Opin. Cell Biol. 9 (2) (1997) 134−142.

[55] C.I. Bargmann, Neurobiology of the *Caenorhabditis elegans* genome, Science 282 (5396) (1998) 2028−2033.

[56] C.J. Matz, P.H. Krone, Cell death, stress-responsive transgene activation, and deficits in the olfactory system of larval zebrafish follwing cadmium exposure, Environ. Sci. Technol. 41 (2007) 5143−5148.

[57] R.P. Oliveira, J.P. Abate, K. Dilks, J. Landis, J. Ashraf, C.T. Murphy, T.K. Blackwell, Condition-adapted stress and longevity gene regulation by *Caenorhabditis elegans* SKN-1/Nrf, Aging Cell 8 (2009) 524−541.

[58] J. Halaschek-Wiener, J.S. Khattra, S. McKay, A. Pouzyrev, J.M. Stott, G.S. Yang, R.A. Holt, S.J. Jones, M.A. Marra, A.R. Brooks-Wilson, D.L. Riddle, Analysis of long-lived *C. elegans* daf-2 mutants using serial analysis of gene expression, Genome Res. 15 (2005) 603−615.


https://doi.org/10.1016/j.heliyon.2019.e01126

[59] S.H. Panowski, A. Dillin, Signals of youth: endocrine regulation of aging in *Caenorhabditis elegans*, Trends Endocrinol. Metabol. 20 (2009) 259–264.

[60] O.J. Marshall, PerlPrimer: cross-platform, graphical primer design for standard, bisulphite and real time PCR, Bioinformatics 20 (15) (2004) 2471–2472.


https://doi.org/10.1016/j.heliyon.2019.e01126