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A cadmium toxicity assay using stress responsive *Caenorhabditis elegans* mutant strains

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ABSTRACT

To test the applicability of *Caenorhabditis elegans* mutant for toxicity screening, the sensitivity of cadmium (Cd) in *C. elegans* was investigated on 14 mutant strains using median lethal concentration (LC50) tests, with further analysis on growth and reproduction conducted on five selected strains. The 24 h LC50 of Cd observed on the wildtype and mutant strains of *C. elegans* was in the order of *age-1*(*hx546*) > *mtl-2*(*gk125*) > *sod-3*(*gk235*) > *daf-21*(*p673*) > *cyp35a2*(*gk317*) > *skn-1*(*or13*) > *daf-12*(*rh62rh157*) > *hsp-16.2*(*gk249*) > *daf-18*(*e1375*) > *ctl-2*(*ok1137*) > wildtype(*N2*) > *sod-1*(*or13*) > *daf-16*(*mu86*) > *cep-1*(*gk138*) > *cdr-2*(*ok1996*). Compared to the wildtype response, a decreased reproduction potential was observed in *mtl-2*(*gk125*), *sod-3*(*gk235*), *cdr-2*(*ok1996*) and *cep-1*(*gk138*) strains. To gain a mechanistic understanding of different sensitivities of the mutant strains, a time-course gene expression analysis was also performed on the five genes. A dramatic increase in the expression of the *mtl-2* gene due to Cd exposure confirmed the importance of this gene in *C. elegans* Cd toxicity. An increased expression of the *sod-3* gene at the longer exposure time period (48 h) suggests that oxidative stress may not be a direct toxic mechanism, but may rather be a consequence of Cd toxicity. Even though, LC50 values for the *age-1*(*hx546*) mutant strain were the highest among the tested strains, the response on the reproduction potential in *age-1*(*hx546*) mutant was unchanged compared to the wildtype, and the *age-1* gene expression remained unaltered on exposure to Cd, which may be interpreted as the maintenance of *age-1* expression level is needed for the exertion of Cd toxicity; however, the role of the *age-1* gene in Cd toxicity may not be via a reproduction-related pathway. The overall results suggest that the *C. elegans* mutant assay seems to be a promising tool for the study of toxic mechanisms, as well as for toxicity screening in ecotoxicological research.

1. Introduction

Global gene expression analysis using microarrays (ecotoxicogenomics) has been increasingly used in ecotoxicology, and offers high sensitivity and mechanistic values in the diagnosis of environmental contamination (Poynton et al., 2007; Roh et al., 2007; Watanabe et al., 2007, 2008). Up- or down-regulation of the genes involved in chemical toxicity can be a good indicator of chemical exposure. However, although microarray-based gene expression provides high sensitivity as an environmental stress response, its application in the field is limited, because it is still very expensive to perform and technically demanding, and most of all is not capable of completely integrating the physiological status of a living organism; thus, has low ecological relevance. Functional genomic tools, such as a mutant, offer the possibility of assessing the physiological meaning of up- or down-regulated gene expression due to chemical exposure and can provide indicators of the toxic mode of action at the single gene level up to that of an entire organism at relatively low cost (Menzel et al., 2007); *Caenorhabditis elegans* is particularly interesting in this respect. The nematode, *C. elegans*, is an excellent model organism for research on and assessment of environmental contaminants, as well as for the study of the ecotoxicological relevance of chemical-induced molecular-level responses (Menzel et al., 2005; Reichert and Menzel, 2005; Leung et al., 2008; Wang et al., 2008). Comprehensive knowledge on the genome of *C. elegans* allows the development of a combination of tests at different organizational levels, such as, at the genome, proteome, organ, organism and population levels (Sochová et al., 2006).

A rich collection of mutant makes *C. elegans* a particularly attractive animal model. The availability of functional genomic tools allows the development of new methods for the sensitive detection of the effects due to chemical exposure. The sensitive mutants can be used to improve the sensitivity of toxic responses and thus, have high potential as toxicity screening tools for chemicals in a relatively short time.

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In this study, the applicability of *C. elegans* mutant for toxicity screening was tested in an ecotoxicological context. Cadmium (Cd) was used as a stressor model, as it is one of the most important toxicant widely found in the environment. Moreover, there have been numerous Cd toxicity studies on *C. elegans* (Barsyte et al., 2001; Ibiam and Grant, 2005; Hughes and Stürzenbaum, 2007). Our laboratory has previously investigated the growth, reproduction and gene expression in *C. elegans* due to metal induced toxicity (Roh et al., 2006). Among four metals studied, Cd showed the most significant results, which lead to deeper study on the toxicity of Cd using mutant strains of *C. elegans*. The fourteen potentially stress sensitive mutants were selected for a toxicity screening study. The sensitivity of *C. elegans* to Cd was screened on these mutant strains using median lethal concentration (LC50) analyses, with further analyses on growth and reproduction conducted on five selected strains. To gain a mechanistic understanding of the different sensitivities of the mutant strains, gene expression analyses were also performed on the five genes.

2. Materials and methods

2.1. Organisms

C. elegans were grown in Petri dishes on nematode growth medium (NGM) and fed OP50 strain *Escherichia coli* according to a standard protocol (Brenner, 1974), with young adults (3 days) from an age-synchronized culture used in all the experiments. To produce age-synchronized cultures, at 2–3 days, eggs from mature adults were isolated using a 10% hypochlorite solution, followed by rinsing with M9 buffer (Hitchcock et al., 1998). The eggs were then allowed to hatch on agar plates with a food source, resulting in synchronized adult worm populations. In addition to wildtype animals (N2 var. Bristol), *mtl-2*(*gk125*), *ctl-2* (*ok1137*), *sod-1*(*tm776*), *sod-3*(*gk235*), *skn-1*(*or13*), *cyp35a2*(*gk317*), *age-1*(*hx546*), *cdr-2*(*ok1996*), *daf-2*(*e1370*), *daf-12*(*rh62rh157*), *daf-16*(*mu86*), *daf-18*(*e1375*), *daf-21*(*p673*), *hsp-16.2*(*gk249*), *cep-1*(*gk138*) mutant strains were used (Supplementary Table 1). Wildtype and mutant strains were provided by the *C. elegans* Genetics Center at the University of Minnesota.

2.2. Lethal toxicity assay

Lethality tests were performed on the wildtype and mutant worms after 24 h of exposure to different concentrations of Cd, as described previously (Roh et al., 2006). Briefly, each test consisted of four concentrations and a control, in which 10 ± 1 young *C. elegans* adults were transferred to 24-well tissue culture plates containing 1 mL of the test solution in each of five wells. The worms were exposed for 24 h at 20 °C. After 24 h, the numbers of live and dead worms were determined via visual inspection by probing with a platinum wire under a dissecting microscope. LC50 were derived through a Probits analysis.

2.3. Growth and reproduction assays

The growth was assessed on the wildtype and mutant worms after 24 h of exposure, as described previously (Roh et al., 2006). Three replicates were conducted for each growth assay. The effects of Cd on the reproduction of wildtype and mutant strains were investigated after 72 h of exposure (Dhawan et al., 1999). After a young adult had been exposed to Cd for 72 h, the number of offspring at all stages beyond the egg was counted. Five replicates were conducted for each reproduction assay.

2.4. Quantitative real-time PCR

C. elegans were exposed to different concentrations of Cd for 12, 24 and 48 h, with total RNA then extracted for PCR analysis according to the standard protocol supplied with the RNeasy Mini kit (Qiagen, Hilden, Germany). An oligo(dT) primer (Bio-Rad laboratories, Hercules, CA, USA) and IQ™ SYBR Green SuperMix (Bio-Rad) were used for the PCR reactions. Quantitative PCR was carried out on five selected genes using a Chromo4 Real-Time PCR detection system (Bio-Rad). The primers were based on sequences retrieved from the *C. elegans* database (www.wormbase.org, Supplementary Table 2). To optimize the qRT-PCR conditions, efficiency and sensitivity tests were performed for each gene prior to the main experiment. Three replicates were conducted for each qRT-PCR analysis.

2.5. Data analysis

Statistical differences between the control and exposed worms were determined using the one-way ANOVA test included in the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA).

Table 1
Estimation of 24 h LC50 in the wildtype(*N2*) and 14 mutant strains of *C. elegans*.

Strains	LC50 (mg/L)	Interval of confidence (95%)
wildtype(*N2*)[a]	846	634 ≤ LC50 ≤ 1064
age-1(*hx546*)	1782	1463 ≤ LC50 ≤ 2120
mtl-2(*gk125*)	1313	1063 ≤ LC50 ≤ 1623
sod-3(*gk235*)	1285	1032 ≤ LC50 ≤ 1599
daf-21(*p673*)	1191	865 ≤ LC50 ≤ 1598
cyp35a2(*gk317*)	1110	893 ≤ LC50 ≤ 1369
skn-1(*or13*)	1028	838 ≤ LC50 ≤ 1241
daf-12(*rh62rh157*)	1006	807 ≤ LC50 ≤ 1334
hsp-16.2(*gk249*)	945	674 ≤ LC50 ≤ 1202
daf-18(*e1375*)	909	582 ≤ LC50 ≤ 1221
ctl-2(*ok1137*)	887	723 ≤ LC50 ≤ 1073
sod-1(*or13*)	796	609 ≤ LC50 ≤ 1049
daf-16(*gk249*)	702	376 ≤ LC50 ≤ 1073
cep-1(*gk138*)	661	508 ≤ LC50 ≤ 869
cdr-2(*ok1996*)	504	285 ≤ LC50 ≤ 714

[a] Roh et al., 2006.

3. Results

The 24 h LC50s for exposure to Cd were examined on the 14 *C. elegans* mutant strains and compared with those of the wildtype. Mutant strains possessing known defective stress response genes were selected; namely, heat shock protein (*hsp-16.2*), metal responsive proteins (*mtl-2*, *cdr-2*), xenobiotic metabolism enzyme (*cyp35a2*), tumor suppressor protein (*cep-1*), aging related protein (*age-1*), antioxidant enzymes (*sod-1-3*, *ctl-2*), antioxidant responsive transcription factor (*skn-1*) and abnormal dauer formation proteins (*daf-12*, *-16*, *-18*, *-21*). The 24 h LC50 for Cd in wildtype *C. elegans* was 846 mg/L (Roh et al., 2006). The 24 h LC50s for Cd exposure in the 14 mutant strains of *C. elegans* were estimated via a Probits analysis (Table 1). The responses of the *mtl-2*(*gk125*), *sod-3*(*gk235*), *skn-1*(*or13*), *cyp35a2*(*gk317*), *age-1*(*hx546*), *daf-12*(*rh62rh157*), *daf-16*(*mu86*) and *daf-21*(*p673*) mutant strains showed greater tolerance/resistance toward Cd exposure than the wildtype. In the *age-1*(*hx546*) strain, the 24 h LC50 was as high as 1782 mg/L. Conversely, the responses of the *cdr-2*(*ok1996*), *cep-1*(*gk138*) and *daf-16*(*mu86*) mutant strains were more sensitive than that of the wildtype strain, with 24 h LC50s of 504, 661 and 702 mg/L, respectively.

Stains more sensitive (i.e. *cdr-2*(*ok1996*) and *cep-1*(*gk138*) mutants) and more resistant (i.e. *mtl-2*(*gk125*), *sod-3*(*gk235*) and *age-1*(*hx546*) mutants) than the wildtype to Cd exposure were subjected to further physiological investigation, using growth and reproduction as the toxic endpoints (Table 2; raw values are presented in Supplementary Table 3). Sublethal exposure concentrations were selected based on the results of the wildtype 24 h LC50 (i.e. 0.85, 8.5, and 85 mg/L; Roh et al., 2006) for exposure conditions for the growth and the reproduction tests. The 24 h growth parameters of *C. elegans* were not significantly affected by exposure to Cd in either the wildtype or mutant strains. However, 72 h reproduction tests revealed that the number of offspring was decreased from about 40 to 75% in the wildtype *C. elegans* on exposure to Cd. The response of the *age-1*(*hx546*) mutant strain was similar to that of the wildtype; whereas, the responses of *mtl-2*(*gk125*), *sod-3*(*gk235*), *cdr-2*(*ok1996*) and *cep-1*(*gk138*) were more sensitive than that of the wildtype (in about 93, 88, 89 and 93%, respectively). A decreased reproduction potential was most important in *cep-1*(*gk138*) and *mtl-2*(*gk125*) strains.

The time course transcriptional expression was analyzed on the five selected genes defected in the studied mutant strains, namely, *age-1*(*hx546*), *mtl-2*(*gk125*), *sod-3*(*gk235*), *cep-1*(*gk138*) and *cdr-2*(*ok1996*), Cd exposed wildtype *C. elegans* (Fig. 1). As expected, Cd exposure induced *mtl-2* gene expression most dramatically (more than 50-fold compared to control); time dependant increases were

Table 2
Ecotoxicological indicators investigated after exposure to Cd in wildtype(*N2*), mutant strains (*cdr-2*(*ok1996*), *cep-1*(*gk138*), *mtl-2*(*gk125*), *sod-3*(*gk235*) and *age-1*(*hx546*)) of *C. elegans*. Results were expressed as the mean value of each parameter compared to wildtype(*N2*) (wildtype(*N2*) = 1; mean ± standard error of mean; number = 5).

Exposure duration	Parameters	Strains	Cd (mg/L)		
			0.85	8.5	85
24 h	Growth	cdr-2(ok1996)	0.95 ± 0.00	0.96 ± 0.02	0.96 ± 0.02
		cep-1(*gk138*)	1.00 ± 0.01	0.97 ± 0.01	0.97 ± 0.01
		mtl-2(*gk125*)	0.98 ± 0.01	0.97 ± 0.01	1.02 ± 0.02
		sod-3(*gk235*)	0.95 ± 0.01	0.95 ± 0.03	0.96 ± 0.01
		age-1(*hx546*)	1.00 ± 0.01	1.01 ± 0.02	1.01 ± 0.05
72 h	Reproduction	*cdr-2*(*ok1996*)	0.51 ± 0.06*	0.51 ± 0.06*	0.52 ± 0.05*
		cep-1(*gk138*)	0.92 ± 0.02	0.34 ± 0.05**	0.30 ± 0.05*
		mtl-2(*gk125*)	1.03 ± 0.03	0.46 ± 0.05**	0.43 ± 0.07*
		sod-3(*gk235*)	1.10 ± 0.03	0.58 ± 0.05*	0.52 ± 0.01*
		age-1(*hx546*)	1.09 ± 0.08	0.78 ± 0.06	0.91 ± 0.08

* $p < 0.05$, ** $p < 0.01$ (compared to wildtye (*N2*)).

observed at two low concentrations tested; whereas, at the highest concentration, no such tendency was observed. Induction of the *sod-3* gene was observed only in *C. elegans* exposed for 48; whereas, this induction occurred in the nematode exposed for 24 at the highest concentration tested (85 mg/L). An increase in *cdr-2* gene expression was observed after 24 and 48 h with exposure to 85 mg/L Cd. The expression of the *age-1* gene was not significantly affected by exposure to Cd.

4. Discussions

The toxic response of *C. elegans* to Cd exposure has been intensively studied (Cui et al., 2007; Hughes and Stürzenbaum, 2007; Dong et al., 2008; Wang et al., 2008), which suggests many biochemical processes and cellular pathways are involved in toxicity and the defense mechanism of *C. elegans* to Cd. Mortality assays were conducted using different mutant strains for the screening of the genes responsible for Cd toxicity or those imparting a protective effect to Cd toxicity. The LC50 observed on the wildtype and mutant strains of *C. elegans* exposed to Cd for 24 h was in the order of *age-1*(*hx546*) > *mtl-2*(*gk125*) > *sod-3*(*gk235*) > *daf-21*(*p673*) > *cyp35a2* (*gk317*) > *skn-1*(*or13*) > *daf-12*(*rh62rh157*) > *hsp-16.2*(*gk249*) > *daf-18* (*e1375*) > *ctl-2*(*ok1137*) > wildtype(*N2*) > *sod-1*(*or13*) > *daf-16*(*mu86*) > *cep-1*(*gk138*) > *cdr-2*(*ok1996*). The LC50 results suggested that defective genes in the mutants having higher LC50 values than wildtype, such as, *age-1*(*hx546*), *mtl-2*(*gk125*), *sod-3*(*gk235*), *daf-21*(*p673*), *cyp35a2*(*gk317*), *skn-1*(*or13*) and *daf-12*(*rh62rh157*) may be responsible to Cd toxicity. Conversely, *cdr-2*(*ok1996*), *cep-1*(*gk138*) and *daf-16*(*mu86*) gene may be involved in the protection to Cd toxicity in *C. elegans*, as the defection of these genes increased the mortality of *C. elegans*. However, no conclusion on the role of genes at the organism level on the responses to Cd exposure can be deduced from only the different susceptibilities on the mortality of the mutant strain. Therefore, growth and reproduction were also investigated on the selected *C. elegans* mutant strains (the three most resistant and two most sensitive mutant strains of those tested), as well as on the wildtype. 24 h Cd exposure did not affect the growth of *C. elegans* in any of the mutant strains or the wildtype. Short term testing; however, only provided a snapshot of the physiological status; thus, longer term testing was conducted to assess the effects of Cd on the reproduction in *C. elegans.* As expected, decreased reproduction potentials were observed in *cdr-2*(*ok1996*) and *cep-1*(*gk138*) strains compared to the wildtype, as they showed higher mortalities in the LC50 test. Surprisingly, however, decreased reproduction toxicity compared to wildtype was also observed, even in the mutant strains that showed a Cd resistant response in the LC50 tests (*mtl-2*(*gk125*), *sod-3*(*gk235*)). These results may suggest that the decline in reproduction is an important cause of mortality in the *cdr-2*(*ok1996*) and *cep-1*(*gk138*) strains, but may not be for the rest of the strains tested. The present study confirmed that Cd exposure seriously affected nematode reproduction, as already observed in our previous study (Roh et al., 2006), where a reproduction test was performed by counting the number of egg inside the transparent worms' body 24 h after exposure. In this study; however, the number of offspring 72 h after exposure was used as a reproduction parameter, which seemed to be more reliable, as it reflected the long term effects on the final reproduction result.

The gene expression analysis, performed to gain a mechanistic understanding of the different sensitivity of mutant strains, revealed dramatic increases in *mtl-2* gene expression and less important increases in *sod-3* gene expression among the five tested genes (Fig. 1). As observed in many previous studies (Swain et al., 2004; Cui et al., 2007; Hughes and Stürzenbaum, 2007; Williams et al., 2009), a dramatic increase in the *mtl-2* gene was also observed due to exposure to Cd, which confirmed the importance of this gene in *C. elegans* Cd toxicity. The strong increase in *mlt-2* gene expression was maintained until 48 h, suggesting *mtl-2* may not only act directly in Cd toxicity, but may also play an important role in response and/or as a defense pathway toward Cd toxicity. The serious decrease in the reproduction potential in the *mtl-2*(*gk125*) mutant strain due to Cd exposure supports this hypothesis.

The increased expressions of the *mtl-2* and *sod-3* genes (Fig. 1) and increased tolerance to Cd exposure in their mutant strains (Table 1) suggested that these genes were responsible for Cd toxicity in *C. elegans*. However, the decreased reproduction potentials of their mutant strains compared to the wildtype (Table 2) were difficult to explain, it can only be deduced that the role of these genes in Cd toxicity may not be via a reproduction-related pathway. Conversely to the *mtl-2* response, no *sod-3* gene expression occurred at an early stage (12 and 24 h), but occurred after a later exposure period (48 h), suggesting that oxidative stress is not a direct toxic mechanism, but may rather be a consequence of progression of toxicity.

The results relating to *age-1*(*hx546*) were particularly interesting, as the expression of this gene was not altered by exposure to Cd, and the response to Cd on the reproduction potential in the *age-1*(*hx546*) mutant strain remained unchanged compared to that in the wildtype, suggesting this gene does not play an important role in Cd toxicity; whereas, the LC50 values of the *age-1*(*hx546*) mutant strain was the highest among the tested strains, which is contradictory to two the other results. The overall results relating to *age-1* may be interpreted as a need for the maintenance of the expression level of this gene in exerting Cd toxicity; however, the role of the *age-1* gene in Cd toxicity may not be via a reproduction-related pathway.

The mutant strains and the toxicity parameters tested in this study were rather limited in terms of allowing a full understanding

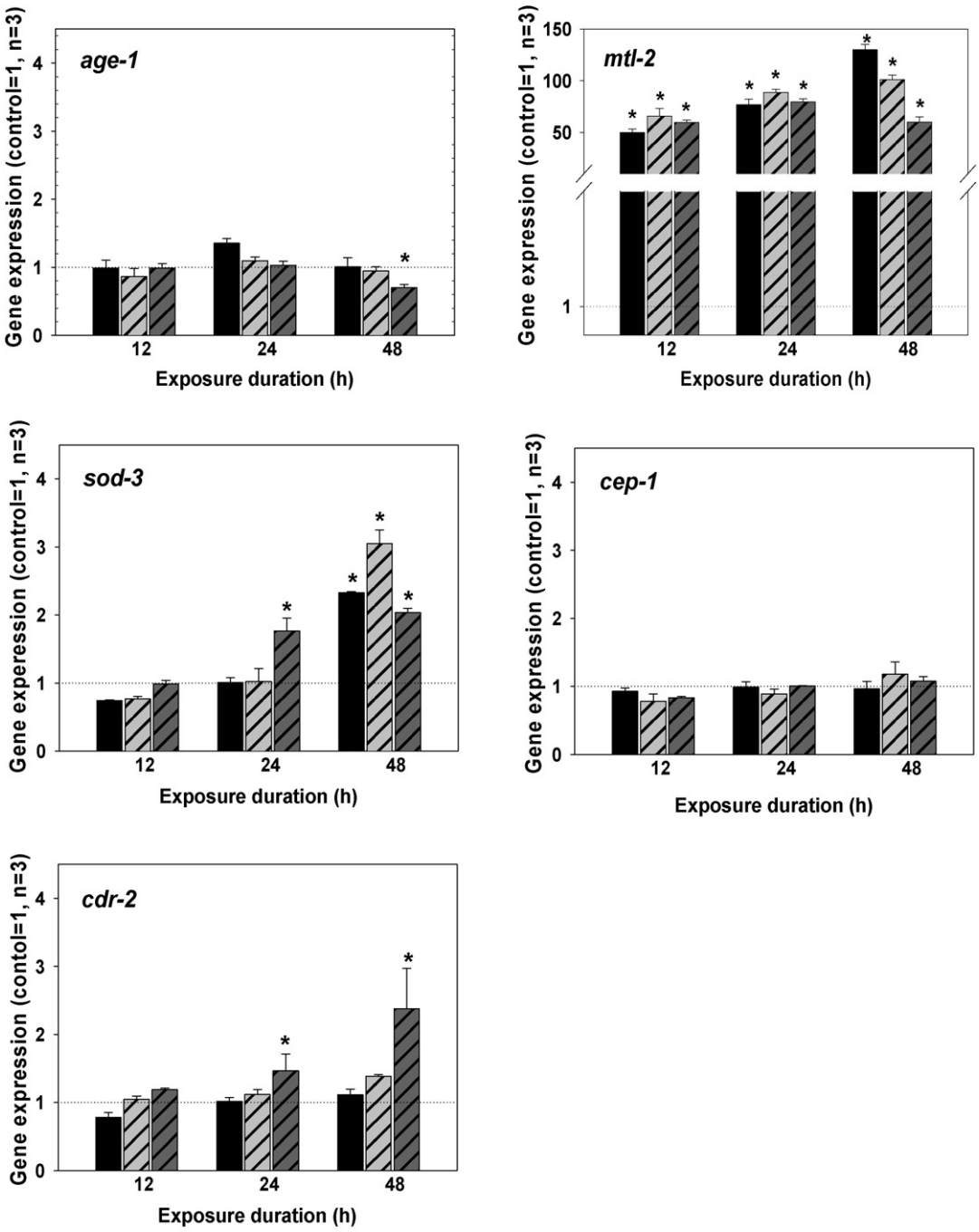

Fig. 1. Cd response gene expression profiling in the young adult of *C. elegans* exposed to Cd for time course (12, 24 and 48 h) using real-time PCR. Densitometric values of stress-related gene expression, normalized using actin mRNA. Data are presented in arbitrary unit compared to control (control = 1, mean ± standard error of mean; number = 3; (■) 0.85, (▨) 8.5, (▩) 85 mg/L) * $p < 0.05$ (compared to control).

of the toxic mechanism and organism response due to exposure to Cd. If further cellular parameters involved in the observed stress response had been tested, the physiological pathway could probably have been more clearly evaluated and explained. Nevertheless, our experiments suggested that the *C. elegans* mutant assay seems to be a promising tool for toxic mechanism, as well as toxicity screening studies in ecotoxicological research, as the possibility of *C. elegans* mutant strain as a bioindicator has previously been reported (Chu et al., 2005). This study suggests that a wide variety of stress responsive *C. elegans* mutant strains can be developed as microarray-like unbiased toxicity screening tools, but in a much simpler and much less expensive way.

Conflicts of interest

We have nothing to declare in any of conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.etap.2009.07.006.

References

Barsyte, D., Lovejoy, D.A., Lithgow, G.J., 2001. Longevity and heavy metal resistance in daf-2 and age-1 long-lived mutants of *Caenorhabditis elegans*. FASEB J. 15, 627–634.

Brenner, S., 1974. The genetics of *Caenorhabditis elegans*. Genetics 77, 71–94.

Chu, K.W., Chan, S.K., Chow, K.L., 2005. Improvement of heavy metal stress and toxicity assays by coupling a transgenic reporter in a mutant nematode strain. Aquat. Toxicol. 74, 320–332.

Cui, Y., McBride, S.J., Boyd, W.A., Alper, S., Freedman, J.H., 2007. Toxicogenomic analysis of *Caenorhabditis elegans* reveals novel genes and pathways involved in the resistance to cadmium toxicity. Genome Biol. 8, R122.

Dhawan, R., Dusenbery, D.B., Williams, P.L., 1999. Comparison of lethality, reproduction, and behavior as toxicological endpoints in the nematode *Caenorhabditis elegans*. J. Toxicol. Environ. Health A 58, 451–462.

Dong, J., Boyd, W.A., Freedman, J.H., 2008. Molecular characterization of two homologs of the Caenorhabditis elegans cadmium-responsive gene cdr-1: cdr-4 and cdr-6. J. Mol. Biol. 376, 621–633.

Hitchcock, D.R., Law, S.E., Wu, J., Williams, P.L., 1998. Determining toxicity trends in the ozonation of synthetic dye wastewaters using the nematode *Caenorhabditis elegans*. Arch. Environ. Contam. Toxicol. 34, 259–264.

Hughes, S., Stürzenbaum, S.R., 2007. Single and double metallothionein knockout in the nematode *C. elegans* reveals cadmium dependent and independent toxic effects on life history traits. Environ. Pollut. 145, 395–400.

Ibiam, U., Grant, A., 2005. RNA/DNA ratios as a sublethal endpoint for large-scale toxicity tests with the nematode *Caenorhabditis elegans*. Environ. Toxicol. Chem. 24, 1155–1159.

Leung, M., Williams, P.L., Benedetto, A., Au, C., Helmcke, K.J., Aschner, M., Meyer, J.N., 2008. *Caenorhabditis elegans*: an Emerging Model in Biomedical and Environmental Toxicology. Toxicol. Sci. 106, 5–28.

Menzel, R., Stürzenbaum, S., Bärenwaldt, A., Kulas, J., Steinberg, C.E., 2005. Humic material induces behavioral and global transcriptional responses in the nematode *Caenorhabditis elegans*. Environ. Sci. Technol. 39, 8324–8332.

Menzel, R., Yeo, H.L., Rienau, S., Li, S., Steinberg, C.E., Stürzenbaum, S.R., 2007. Cytochrome P450s and short-chain dehydrogenases mediate the toxicogenomic response of PCB52 in the nematode *Caenorhabditis elegans*. J. Mol. Biol. 370, 1–13.

Poynton, H.C., Varshavsky, J.R., Chang, B., Cavigiolio, G., Chan, S., Holman, P.S., Loguinov, A.V., Bauer, D.J., Komachi, K., Theil, E.C., Perkins, E.J., Hughes, O., Vulpe, C.D., 2007. *Daphnia magna* ecotoxicogenomics provides mechanistic insights into metal toxicity. Environ. Sci. Technol. 41, 1044–1050.

Reichert, K., Menzel, R., 2005. Expression profiling of five different xenobiotics using a *Caenorhabditis elegans* whole genome microarray. Chemosphere 61, 229–237.

Roh, J.Y., Lee, J., Choi, J., 2006. Assessment of stress-related gene expression in the heavy metal-exposed nematode *Caenorhabditis elegans*: a potential biomarker for metal-induced toxicity monitoring and environmental risk assessment. Environ. Toxicol. Chem. 25, 2946–2956.

Roh, J.Y., Jung, I.H., Lee, J.Y., Choi, J., 2007. Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode *Caenorhabditis elegans*. Toxicology 237, 126–133.

Sochová, I., Hofman, J., Holoubek, I., 2006. Using nematodes in soil ecotoxicology. Environ. Int. 32, 374–383.

Swain, S.C., Keusekotten, K., Baumeister, R., Stürzenbaum, S.R., 2004. *C. elegans* metallothioneins: new insights into the phenotypic effects of cadmium toxicosis. J. Mol. Biol. 341, 951–959.

Wang, S., Tang, M., Pei, B., Xiao, X., Wang, J., Hang, H., Wu, L., 2008. Cadmium-induced germline apoptosis in *Caenorhabditis elegans* the roles of HUS1, p53, and MAPK signaling pathways. Toxicol. Sci. 102, 345–351.

Watanabe, H., Kobayashi, K., Kato, Y., Oda, S., Abe, R., Tatarazako, N., Iguchi, T., 2008. Transcriptome profiling in crustaceans as a tool for ecotoxicogenomics: *Daphnia magna* DNA microarray. Cell Biol. Toxicol. 24, 641–647.

Watanabe, H., Takahashi, E., Nakamura, Y., Oda, S., Tatarazako, N., Iguchi, T., 2007. Development of a *Daphnia magna* DNA microarray for evaluating the toxicity of environmental chemicals. Environ. Toxicol. Chem. 26, 669–676.

Williams, P.L., Ma, H., Glenn, T.C., Jagoe, C.H., Jones, K.L., 2009. A transgenic strain of the nematode *Caenorhabditis elegans* as a biomonitor for heavy metal contamination. Environ. Toxicol. Chem., doi:10.1897/08-496.1.