

Gene Expression Profiling in the Nematode *Caenorhabditis elegans*, as a Potential Biomarker for Soil Ecotoxicology

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잠재적 생체지표 발굴을 위한 토양선충 *Caenorhabditis elegans*에서의 유전자 발현 연구

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ABSTRACT

Monitoring toxicity levels in specific biological compartments is necessary to evaluate the ecotoxicological risk associated with soil environmental pollution. Gene expression, as potential biomarker, is increasingly used as rapid early warning systems in environmental monitoring and ecological risk assessment procedures. Various representative species are currently used for the purpose of assessing soil toxicity, however, investigations on toxicological assessments using endpoint based on gene-level have been limited. In this review, we will present the current trends in organisms and endpoints used in soil toxicity study and report gene expression related to toxicity using soil organism, and *C. elegans* as promising organisms for this approach.

Key words : gene expression profiling, *Caenorhabditis elegans*, soil ecotoxicology

INTRODUCTION

Soil ecotoxicology is a scientific discipline for effort to protect soil by studying the relationships between the contaminated soil and effects on representative soil organisms. Soil organisms perform various ecological functions that reflect the ecological stability of land. Their functions are influenced by soil quality, on the other hand, they give advance warning of risk from environmental pollutant to human life. A wide

variety of soil organisms has been used for both approaches including microorganisms, earthworms, enchytraeids, springtails, mites, and insects (Løkke and van Gestel, 1998; Cortet *et al.*, 1999). Especially, invertebrate are good biological models for soil studies, because their population are often abundant and their life cycles are frequently short. Therefore, samples can be taken for analysis without significantly affecting population dynamics and population level effects can be examined concomitantly with the response of biomarkers. And though their permeable cuticle they are in direct contact with xenobiotics dissolved in interstitial water which may have adverse effects. Regarding their important contribution to the soil

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Table 1. The representative species for soil ecotoxicity monitoring

	Species	References
Earth worm	<i>Perionyx excavatus</i>	De Silva and van Gestel, 2009
	<i>Eisenia andrei</i>	Hurdzan and Lanno, 2009
	<i>Eisenia fetida</i>	Fernández <i>et al.</i> , 2009
	<i>Enchytraeus albidus</i>	Loureiro <i>et al.</i> , 2009
	<i>Eisenia veneta</i>	Fausser <i>et al.</i> , 2005
	<i>Lumbricus rubellus</i>	Andre <i>et al.</i> , 2009
	<i>Lumbricus terrestris</i>	Kobetičová <i>et al.</i> , 2008
Enchytraeid worm	<i>Enchytraeus crypticus</i>	Scott-Fordsmand <i>et al.</i> , 2008
Springtail	<i>Orchesella cincta</i>	Janssens <i>et al.</i> , 2007
	<i>Folsomia candida</i>	Bur <i>et al.</i> , 2010
Woodlouse	<i>Porcellio scaber</i>	Udovic <i>et al.</i> , 2009
Nematode	<i>Caenorhabditis elegans</i>	Höss <i>et al.</i> , 2009

environment, the safety of soil invertebrates inhibited in contaminated soil should be monitored carefully.

Ecotoxicological monitoring using organisms has been devoted to study the effects of environmental contaminants at the population, community or ecosystem levels (Forbes and Forbes, 1994). However, these traditional approaches are sometimes inefficient, especially to adequately assess the effects of chronic exposure of organisms at low levels of xenobiotics and to detect early biological responses. Therefore, rapid and sensitive tools are needed for screening hazardous properties of environmental pollutant prior to intensive toxicological investigation and risk assessment. There has been a shift in emphasis towards understanding the sublethal effects of long-term exposure to contaminants at the individual level where exposure can be adequately described and assessed (Newman and Jagoe, 1996). Therefore, the understanding of the effects of toxicants at the molecular or biochemical levels may provide some insights into the cause of effects identified at higher level (Newman and Unger, 2003). Discovery of biomarkers using molecular biological tools, gene expression, has been gaining popularity as 'early warning' tools for monitoring environmental quality and detecting environmental contamination.

This review will present current trends in soil organism and endpoints used in soil toxicity study. Also, the use of gene expression profiling as potential biomarker in soil organism will be discussed in the con-

text of environmental toxicological monitoring. Special focus will be made on the model organism, the nematode *Caenorhabditis elegans*.

CURRENT TRENDS IN ORGANISMS AND ENDPOINTS USED IN SOIL TOXICITY STUDY

To predict the effects of pollutants on soil invertebrate communities, information must be extrapolated from a representative species, because it is impractical to conduct a large number of tests, where the diversity of soil fauna is not precisely known (Frampton *et al.*, 2006). Various representative species are currently used for the purpose of assessing soil toxicity (Table 1). For decades, standardized soil toxicity tests have been carried out using primarily many earthworm species with the most common endpoints of survival, reproduction, fertility and behavior. As standard laboratory test methods, an acute test for assessing mortality and chronic tests for assessing reproduction are currently available for several test organisms (OECD, 1984, 2004; ISO, 1993, 1998, 2006).

Of the invertebrates used for ecotoxicity the assessment of ecotoxicity, the earthworm *Eisenia fetida* and springtail *Folsomia candida* have been most widely used in field applications as well in laboratory investigation (Fig. 1A).

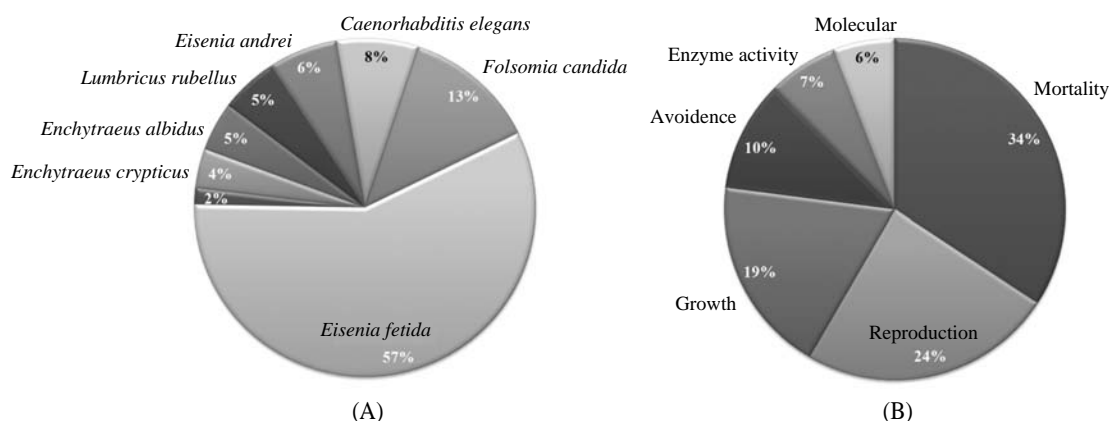


Fig. 1. Summary of current use of soil organisms in ecotoxicity study (A). This data was organized with research paper which published during the years 2000 to 2009 in “Pubmed” using keywords “soil toxicity” and subsequently each organism name. Total number of used data for this figure is 631 (*Eisenia fetida*, 375; *Folsomia candida*, 76; *Caenorhabditis elegans*, 37; *Eisenia andrei*, 34; *Lumbricus rubellus*, 31; *Enchytraeus albidus*, 32; *Enchytraeus crypticus*, 23; Others: *Porcellio scaber*, *Meloidogyne javanica*, *Panagrellus redivivus*, *Eisenia veneta*, *Orchesella cincta*, *Pratylenchus penetrans*, 23). Summary of current endpoints used for ecotoxicity study in soil organism (B). This data was organized with research paper which published during the years 2006 to 2009 in “Pubmed” using keywords “soil toxicity” and each organism name. Total number of availability data is 274 (mortality, 88; reproduction, 62; growth, 48; avoidance, 27; enzyme activity, 17; subcellular, 15).

As toxic endpoints, individual level endpoints, such as, survival, reproduction growth and development have mainly been investigated. However, investigations on toxicological assessments using endpoint based on cellular-level, such as, gene and protein expression and enzyme activity, have been limited (Fig. 1B). In soil ecosystems, chemical pollution is frequently caused by a complex mixture of contaminants rather than a single contaminant. Toxicities associated with exposure to chemical mixtures are often difficult to delineate due to the complexity of both the characteristics of the mixture itself, the chemical-to-chemical differences in toxicokinetic and mechanism(s) of toxic action. Therefore, emphasis on development of biomarker using multiple endpoints in soil organism should be taken. The use of biomarkers has been reported to be very informative about the organism’s stress response to individual toxicants and mixtures (Steinberg *et al.*, 2008; Menzel *et al.*, 2009). Such results indicate that research of sensitive biomarkers at molecular and biochemical level in soil toxicity monitoring works is extremely important. According to Steinberg *et al.*

(2008), the traditional ecotoxicological approach superficial and more chemical compound oriented is not toxic mechanisms orientated. However, gene expression is recognized as a useful biomarker of exposure because of its high sensitivity. Gene expression is involved in the initial stages of stress responses and is responsive at low doses of exposure, even at levels, thus at which toxic effects are not observed.

GENE EXPRESSION; POTENTIAL BIOMARKER IN SOIL ORGANISMS

Gene expression profiling in a wide variety of organisms has become technically feasible in the last decade (Gibson, 2002). Even though the gene expression profiles are altered during toxicity, as either a direct or indirect result of toxicant exposure, the comparison of numerous specific expression profiles facilitates the differentiation between homeostasis and true responses to environmental stressors (Steinberg *et al.*, 2008). Furthermore, the application of gene expression

result provides the means to identify complex pathways that an exposed organism applies in response to environmental stressors. Gene expression profiling, therefore, represents a powerful endpoint for ecotoxicology and is a means for bringing the genomics revolution to this field. Progress in the molecular description of model organisms has produced knowledge that is generalizable to the non-model organisms of interest to ecotoxicology. Rapidly growing sequence databases are providing genetic tools for dissecting stress responses in many species, even those for which little sequence is available (Table 2).

Among soil invertebrates, *F. candida* (www.collem-base.org) and *L. revellus* (xyala.cap.ed.ac.uk/Lumbri-base) have been the subject of an expressed sequence tag (EST) sequencing project, permitting the construction of cDNA microarrays. *F. candida* is the most intensively investigated springtail species in a broad range of disciplines including, ecotoxicology, physiology, genetics, genomics and many more. In addition, the gene expression of *F. candida* in response to stress has been used to evaluate the toxicity of pollutants. Thus, some gene expressions of springtails following contaminated soil exposure have been used as biomarker (i.e., the variation of metallothionein gene expression in *F. candida* exposed to cadmium; Nota *et al.*, 2008; Nakamori *et al.*, 2009).

L. rubellus is also a common temperate earthworm, used as a 'model' species by many researchers and others working on the biology and ecology of the soil. A large scale genome sequencing project for this species has started in Edinburgh, UK. Investigation of metallothionein expression on *L. rubellus* have been conducted on cadmium, copper, lead, mercury and nickel (Nadeau *et al.*, 2001; Sturzenbaum *et al.*, 2004; Burgos *et al.*, 2005; Spurgeon *et al.*, 2005). Another representative organism for soil ecotoxicological monitoring is *E. fetida*, commonly known as red wiggler or compost worm, belongs to the Lumbricidae family of the Annelida phylum. Little is known about its genome sequence although it has been extensively used as a test organism in terrestrial ecotoxicology. Wilding *et al.*, reported that the successful Expressed

Sequence Tag (EST) project is available for the earthworm *L. rubellus* it is possible to develop PCR assay to target any region of interest in the mtDNA utilising sequence information (Wilding *et al.*, 2006). However, the applications of gene expression as a biomarker in environmental diagnosis or assessment are still very limited.

As shown in Table 3, some genes have been identified as potential biomarkers for assessing toxicity. In Table 3, potential biomarkers discovered from various representative soil species for the assessment of chemical risk are listed. The results were compared with those obtained with *C. elegans*.

CAENORHABDITIS ELEGANS AS A GOOD MODEL SYSTEM FOR CHEMICAL-INDUCED GENE EXPRESSION AND FUNCTIONAL TOXICOGENOMICS

C. elegans is a ubiquitously distributed free-living nematode that lives mainly in the liquid phase of soils. It is the first multicellular organism to have its genome completely sequenced. The genome showed an unexpectedly high level of conservation relative to the vertebrate genome, which makes *C. elegans* an ideal system for biological studies in areas such as, genetics, and molecular and developmental biology (Cutter *et al.*, 2009). Recently, in addition to its abundance this species has also been used as an animal model for ecotoxicological studies, and due to its sensitivity to various types of environmental contaminants, as well as its feasibility in the experimental approach to chemical-induced molecular-level responses (Table 4).

C. elegans has several advantages over other species in gene expression analysis. WormBase (Harris *et al.*, 2004), the information-rich central genomic database of *C. elegans*, provides an intuitive interface into a well-annotated genome. *C. elegans* also has a consistent system of gene identification, thereby avoiding the confusion of gene identification that is common

Table 2. Examples of gene expression analysis in soil organisms

Organisms	Target gene	Chemical	Media	References
<i>Orchesella cincta</i>	metallothionein	Pb, Zn, Cu, Cd, Fe, Ca	metal contaminated soil	Timmermans <i>et al.</i> , 2005
<i>Metaphire posthuma</i> , <i>Polypheretima elongata</i>	metallothionein	Cd	spiking soil	Liang <i>et al.</i> , 2009
<i>Lumbricus terrestris</i>	hsp	Pb, Cd, Cu, Hg	AS*	Nadeau <i>et al.</i> , 2001
<i>Lumbricus rubellus</i>	metallothionein	Cd	spiking soil	Morgon <i>et al.</i> , 2004
	mt-2, l-rRNA, lgp	Cd, Cu	spiking soil	Spurgeon <i>et al.</i> , 2004
	mt, aox, lys	Cu, Cd	AS*	Burgos <i>et al.</i> , 2005
	mtl-1, 2	Cd, Cu, Pb, Zn	field soil site	Spurgeon <i>et al.</i> , 2005
	mt	metal	metalliferous soils	Spurgeon <i>et al.</i> , 2006
<i>Folsomia candida</i>	Expressed gene tag	Cd, Cu, atrazine, Fluoranthene		Owen <i>et al.</i> , 2007
	cDNA microarray	Cd, FA, AZ	spiking soil	Svendsen <i>et al.</i> , 2008
	microarray	Cd	lufa	Nota <i>et al.</i> , 2008
	microarray	PAH	lufa soil	Nota <i>et al.</i> , 2009
<i>Eisenia fetida</i>	cadmium-responsive gene	Cd	AS*	Nakamori <i>et al.</i> , 2009
	<i>NF-κB</i> , <i>hsp</i> , <i>MapKK</i> , <i>ATPase</i> , <i>Aqua</i> , <i>ACR</i> , <i>MSH</i>	Temperature, pH	AS*	de Boer <i>et al.</i> , 2009
	<i>Microarray</i>	heat stress	Lufa	Nota <i>et al.</i> , 2010
	annetocin gene	Pb, Zn	metalliferous soil	Ricketts <i>et al.</i> , 2004
<i>Eisenia fetida</i>	mt, cat, hsp, bark, calm, pkc, tetp, sod	Cd	AS*	Brulle <i>et al.</i> , 2008
	metallothionein	Cd	filter paper	Demuyneck <i>et al.</i> , 2006
	metallothionein	Cd, Zn	spiking soil	Demuyneck <i>et al.</i> , 2007
	microarray	2,4,6-trinitrotoluene	spiking soil	Gong <i>et al.</i> , 2007
	TCTP	PAH	AS*	Zheng <i>et al.</i> , 2008

* AS indicates artificial soil.

Table 3. Representative potential biomarker investigated on various different terrestrial organisms. This data was organized with research paper in “pubmed”, “sciencedirect” and “google”

Biomarker	<i>Lumbricus rubellus</i>	<i>Folsomia candida</i>	<i>Eisenia fetida</i>	<i>Caenorhabditis elegans</i>
Metallothionein The number of Cd ions fixed by a molecule of MT. MT-2 is more responsive to cadmium than copper.	✓	✓	✓	✓
Cadmium response gene cdr-1 is transcribed in intestinal cells in response to cadmium exposure.				✓
Phytochelatin phytochelatin were formed from glutathione in the presence of cadmium ions by an they characterized as r-glutamylcysteine dipeptidyl transpeptidase.	✓	✓	✓	✓
Selenoprotein The occurrence of selenoproteins in selenium-sensitive plants may therefore be an excellent biomarker for selenium-induced stress in some plants.		✓		✓
Vitellogenin Vitellogenins (Vg) are the major precursor of the egg-yolk proteins, which are generally synthesised in response to endogenous estrogens.	✓	✓		✓
Estrogen-binding proteins The nonylphenol significantly inhibited receptor binding of estrogen to one of the nematodes.				✓
Acetylcholinesterase response gene AChE inhibition was used to test a field population of <i>P. clarkii</i> potentially exposed to high concentrations of the organophosphorus pesticide fenitrothion.	✓	✓	✓	✓
Cytochrome P450 The use of P450 induction as biomarker of exposure to pollutants has been well described in several organisms, vertebrates and invertebrates.	✓	✓		✓
Glutathione S-transferases Gst must be a mechanism similar to that of P450. That could be implicated in resistance of insects to insecticides.	✓	✓		✓

in many species, including human (Leung *et al.*, 2008).

Although gene expression as environmental stress-response provides high sensitivity and thus can act as an early warning signal, relating such a molecular level response to ecological effects represents a substantial challenge that can only be met by a large-scale investigation at different biological organizations (molecular, individual organism and population level). A link or correlation between a conventional toxicity endpoint (e.g., growth reproduction and mortality)

and upstream-induced gene expression is interesting, particularly for ecotoxicological purposes. Functional genomic tools, such a mutant or RNAi, offer the possibility of assessing the physiological meaning of up- or down-regulated gene expressions due to chemical exposure. Also these tools 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). A rich collection of mutant makes *C. elegans* a particularly attractive animal model. Sensitive mu-

Table 4. Gene expression profiling analysis available for *Caenorhabditis elegans* model

Chemical	Endpoints (Target genes)	References
heavy metal	stress response gene	Roh <i>et al.</i> , 2006
heavy metal	RNA/DNA ration	Ibiam and Grant, 2005
Cd	Microarray	Cui <i>et al.</i> , 2007
Zn, Cd, As, Hg	<i>Cdr</i>	Dong <i>et al.</i> , 2008
chlorpyrifos	stress response gene	Roh <i>et al.</i> , 2007
paraquat	<i>daf</i>	Kim <i>et al.</i> , 2007
DEHP	Microarray	Roh <i>et al.</i> , 2008
b-NF, Fla, atrazine, clofibrate, DES	<i>cytochrome P450, gst, UDPGT</i>	Reichert and Menzel, 2005
Allyl isothiocyanate	<i>gst</i>	Hasegawa <i>et al.</i> , 2010
octachlorostyrene	Whole gene profiling	Kim and Choung, 2009
silver nano	Microarray, <i>pmk-1</i>	Roh <i>et al.</i> , 2009
PCB52	<i>cyp</i>	Menzel <i>et al.</i> , 2007
organophosphorus pesticides	Microarray	Lewis <i>et al.</i> , 2009
zinc oxide nano	<i>mtl</i>	Ma <i>et al.</i> , 2009
river sediments	Microarray	Menzel <i>et al.</i> , 2009

tants can be used to improve the sensitivity of toxic response and thus have high potential for screening a toxicity of chemicals in a relatively short time (Chu *et al.*, 2005). Previously, we reported that a wide variety of stress responsive in *C. elegans* mutant strains can be developed as microarray-like unbiased toxicity screening tools (Roh *et al.*, 2009), but in a much simpler and less expensive way. Mutant *C. elegans* can be used to confirm the role of specific molecular targets based on gene expression analysis (Menzel *et al.*, 2007). We also used *C. elegans* RNAi technique to investigate toxic mechanism of nanoparticles in ectotoxicological context (Roh *et al.*, 2010).

CONCLUSION REMARKS

Xenobiotically induced gene expression is considered a highly promising tool in biomonitoring for the early detection of environmental contaminants. Gene expression endpoints are not only sensitive and useful in estimating the effects of toxicants on expected populations, but may also provide an insight into the mechanisms underlying these effects. However, their field application is still limited, because these systems are not capable of completely integrating the physiological status of a living organism, and thus, have low

ecological relevance. While the biological parameters from higher levels of biological organizations, such as growth and reproduction, are accepted as valid and standardized endpoints, their responses are nonetheless not very sensitive or specific. The main advantage of the gene expression test compared to growth or reproduction test is the increased sensitivity and specificity. The parallel determination of a variety of stress-inducible genes (e.g., by using DNA microarray) will considerably promote the implementation of this approach. Depending on the selected marker genes, this approach has the potential of identify substance class-specific effects. What is needed, in the future, are new and innovative approaches that integrate effects across different levels of biological complexity and provide a clear understanding of all the hazards posed by environmental pollution. *C. elegans* seems to be a powerful model for this approach. Especially, the comprehensive knowledge of the genome of *C. elegans* and functional genomic tools allow the development of new methods for the sensitive detection of the effects of pollutant exposure.

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