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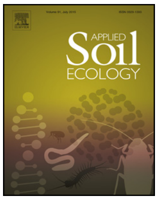

Earthworm toxicogenomics: A renewed genome-wide quest for novel biomarkers and mechanistic insights☆

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ABSTRACT

As sentinel species, earthworms such as *Eisenia* spp. and *Lumbricus* spp. have been considered among the best bioindicators or biomonitors for soil ecosystems owing to their close contact with the environment and essential roles in soil pedogenesis, structure, fertility and the terrestrial food chain. Earthworms have also been used extensively for assessing environmental risk and chemical toxicity in laboratory and field settings. In the past two decades, a comprehensive set of transcriptomic, proteomic, metabolomic and bioinformatic tools have been developed and applied to assess ecological impacts of contaminated soils on earthworms. In this review, we summarize recent progress made in earthworm toxicogenomics, with an emphasis on earthworm toxicotranscriptomics, examine novel biomarkers discovered and mechanistic insights gained through toxicogenomics studies, discuss currently existing technical hurdles to be resolved in order to move ahead, and finally provide some remarks on the future perspectives of this interdisciplinary and promising field.

1. Introduction

Changes in environmental quality and health can be perceived by soil invertebrates living in direct contact with the environment (Gong et al., 2012a). In contrast to terrestrial vertebrates that are exposed to xenobiotics primarily through the food chain, soil-dwelling invertebrates such as nematodes, isopods, springtails (Collembola), gastropods and oligochaetes are exposed through a variety of routes like ingestion, burrowing, dermal contact and feeding on other organisms (Kammenga et al., 2000; Weeks et al., 2004; Sutekova and Hofman, 2011). Many of these invertebrates are sensitive to soil contaminants and are valuable sentinel species or bioindicators. In choosing terrestrial bioindicator species, the following features are often considered: toxicological sensitivity, ecological importance and representativeness, facility to be maintained in laboratory, reproductive rate, and ease in field collection, identification and analysis (Greenslade, 2007; Roembke and Garcia, 2002; Fontanetti et al., 2011).

As sentinel species, earthworms such as *Lumbricus* spp. and *Eisenia* spp. have been considered among the best bioindicators for monitoring the health of terrestrial ecosystems (Hirano and Tamae, 2011) owing to their vital roles in soil pedogenesis, soil structure, fertility and terrestrial food chain (Edwards, 2004). As a result, earthworms play an important role in terrestrial ecotoxicology where they are used extensively for assessing environmental risk and chemical toxicity in both laboratory and field settings (Sanchez-Hernandez, 2006). Standardized protocols and guidelines for earthworm acute toxicity, reproductive toxicity, avoidance behavior and field testing have been developed and/or adopted by such international organizations as Organization of Economic Cooperation and Development (OECD), International Organization for Standardization (ISO) and American Society for Testing and Materials (ASTM), as well as national regulatory or legislative agencies such as U.S. Environmental Protection Agency (EPA), Environment Canada (EC) and European Union (EU). There also exists a large and growing body of earthworm toxicity datasets in various ecotoxicological databases. For instance, U.S. EPA's ECOTOX/TERRETOX database (www.epa.gov/ecotox) currently has 3369 records of toxicity studies using *Eisenia fetida* as the testing organism.

The measurement of gene/protein expression and metabolite levels, upon exposure to a chemical or a stressor, can be used to develop robust molecular biomarkers for detection of environmental stress, in the study of long-term exposure impacts, and for inference of mechanisms of action (Calzolai et al., 2007; Snape et al., 2004). The use of biomarkers in soil invertebrates (including earthworms) for ecological risk assessment has gained a significant

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degree of popularity over the past several decades. A biomarker can be defined as a xenobiologically induced variation in molecular, cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample (Edwards, 2004). A wide variety of biochemical, physiological and molecular earthworm biomarkers have been investigated (Scott-Fordsmand and Weeks, 2000), including DNA alteration, induction of metal-binding proteins (*e.g.*, metallothionein), depression of enzymatic activities (*e.g.*, cholinesterase, cytochrome P-450, glutathione *S*-transferase, peroxidase and catalase), energy reserve responses, and changes in immunology, lysosomal membrane stability, neural impulse conductivity, sperm counts and quality, histopathology, and behavior. Several major efforts have examined a number of these biomarkers, such as the EU-supported BIOPRINT-I and II projects (Sutekova and Hofman, 2011; Weeks et al., 2004). Nevertheless, when applied separately, these biomarkers only examine one or a small number of biological processes in a complex biological system and have limited power in distinguishing chemical-specific responses. They are not suitable for assessing a wide variety of contaminants, particularly those chemicals with unknown modes of action (MOAs).

In the wave of recent revolutionary advances in high-content omics technologies represented by DNA microarray (Welle, 2013) and massively parallel DNA sequencing (van Dijk et al., 2014), a renewed quest for novel biomarkers and insights into toxicological MOAs has been initiated within the scope of an emerging discipline called earthworm toxicogenomics. Toxicogenomics is a field of science that deals with the collection, interpretation, and storage of information about gene, protein and metabolic activity within a particular cell or tissue of an organism in response to toxic substances (National Research Council, 2005). It meshes toxicology with genomics or molecular profiling, *i.e.* transcriptomics, proteomics and metabolomics (Hamadeh and Afshari, 2004; National Research Council, 2005). As a sub-discipline of ecotoxicogenomics that integrates genomics with ecotoxicology (Snape et al., 2004), earthworm toxicogenomics is defined herein as application of transcriptomic, proteomic and metabolomic approaches to earthworm toxicology centered around the discovery of novel biomarkers and elucidation of ecotoxicological mechanisms, or MOAs.

2. An overview of earthworm toxicogenomics studies

In this review, we adopt the following definitions for omics approaches:

- Transcriptomics is the study of the transcriptome (*i.e.*, the complete set of RNA transcripts that are produced by the genome under specific circumstances or in a specific cell) using microarray, next generation sequencing (NGS) and other high-throughput technologies (Dong and Chen, 2013).
- Proteomics is the large-scale study of the proteome, the complete set of proteins expressed by an organism, tissue, or cell, particularly the changes in protein abundance, structure, function, expression pattern, and interactions as related to diseases and environmental conditions (Graves and Haystead, 2002).
- Metabolomics is the analysis of endogenous and exogenous low molecular mass metabolites within a cell, tissue, or biofluid of an organism in response to an external stressor (Lankadurai et al., 2013b).

Comparison of transcriptomes, proteomes and metabolomes allows the identification of genes, proteins and metabolites that are differentially regulated in distinct cell populations, or in response to different treatments.

Our literature survey for earthworm toxicogenomics identified 59 studies over the last 18 years (1997–2014) (Table 1). Two-thirds of these studies (39/59) were published in past five years. Present in these were 21 transcriptomic, 5 proteomic and 35 metabolomic studies, among which two studies employed a dual omics approach, *i.e.*, an integration of transcriptomics or proteomics with metabolomics (Bundy et al., 2008; Ji et al., 2013). A broad spectrum of toxicants have been examined, ranging from metals (*e.g.*, As, Cd, Cu, Pb, Ti and Zn) and organics (PCBs and PAHs) to insecticides (carbaryl, carbofuran, chlorpyrifos, DDT, and endosulfan), herbicide (atrazine), fungicide (epoxiconazole), natural toxin (2-phenylethyl isothiocyanate), flame retardants (Dechlorane Plus and BDE 47), pharmaceutical drugs (carbamazephine and estrone), chemical warfare agents (mustard and nerve agent), and explosives (TNT, RDX, HMX and CL-20). Exposure scenarios varied from chemical-amended soil, filter paper or solutions under laboratory conditions to naturally contaminated soils under laboratory (*ex situ*) or field (*in situ* mesocosom) conditions. The length of exposure ranged from 48-hr in acute dermal contact tests to many years in field chronic studies. In addition to chemical stressors, the response of earthworms to cold shock (Kim et al., 2012) and pathogen stress (*Escherichia coli* O157:H7, (Wang et al., 2010b, 2011) has been examined.

We have also identified six review articles with the broad scope of environmental toxicogenomics, in which earthworm toxicogenomics was partially covered. Three of them reviewed earthworm transcriptomics (Brulle et al., 2010; van Straalen and Roelofs, 2008; Vasseur and Bonnard, 2014), whereas the other three earthworm metabolomics (Hernandez-Soriano and Jimenez-Lopez, 2014; Lankadurai et al., 2013b; Simpson and McKelvie, 2009). Two of the earthworm transcriptomics reviews (Brulle et al., 2010; van Straalen and Roelofs, 2008) were limited in scale and depth. They only focused on interpretation of published studies and discussed such subjects as the advantages of transcript profiling over traditional bioassays in terms of specificity, sensitivity and rapidity (van Straalen and Roelofs, 2008) as well as the contributions of transcriptomics to our understanding of earthworm toxicity at the molecular level (Brulle et al., 2010). Vasseur and Bonnard (2014) touched the base of earthworm transcriptomic response to toxicants and concentrated instead on genotoxicity biomarkers such as DNA adducts and DNA breakage as well as other genetic and epigenetic biomarkers like AFLP (amplified fragment length polymorphism) and DNA methylation in earthworms. The latter three reviews provided comprehensive overviews of the experimental design, extraction methods, analytical instrumentation, and statistical methods used in environmental metabolomics, and highlighted some recent earthworm metabolomics studies to elucidate hitherto unknown biochemical modes of actions of environmental stressors, in addition to identifying metabolite shifts as potential early bioindicators of stressors. To avoid repetition, we do not intend to revisit these topics in the present review.

In the past five years, significant progress has been made in applying NGS technologies (Zhang et al., 2014) and integrated omics approaches (*e.g.*, transcriptomics + metabolomics (Bundy et al., 2008), proteomics + metabolomics (Ji et al., 2013)) to earthworm toxicogenomics. New bioinformatic infrastructure and computational methodologies have also been developed, specifically for earthworm toxicogenomic research. Here, we will focus our discussions on these new developments as well as integrating different omics approaches, a topic not covered in previous reviews. The objectives of this review are to (a) summarize recent progress in earthworm toxicogenomics, with an emphasis on earthworm toxicotranscriptomics; (b) examine novel biomarkers discovered and mechanistic insights gained through toxicogenomics studies; (c) discuss technical hurdles that

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