ELSEVIER

Chemosphere 61 (2005) 229–237

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

# Expression profiling of five different xenobiotics using a *Caenorhabditis elegans* whole genome microarray

Kerstin Reichert [a,*], Ralph Menzel [b]

[a] *Institute of Biology, Ecotoxicology and Biochemistry, Free University Berlin, Ehrenbergstrasse 26-28, 14195 Berlin, Germany*
[b] *Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany*

Received 28 October 2003; received in revised form 7 December 2004; accepted 21 January 2005
Available online 10 March 2005

**Abstract**

The soil nematode *Caenorhabditis elegans* is frequently used in ecotoxicological studies due to its wide distribution in terrestrial habitats, its easy handling in the laboratory, and its sensitivity against different kinds of stress. Since its genome has been completely sequenced, more and more studies are investigating the functional relation of gene expression and phenotypic response. For these reasons *C. elegans* seems to be an attractive animal for the development of a new, genome based, ecotoxicological test system. In recent years, the DNA array technique has been established as a powerful tool to obtain distinct gene expression patterns in response to different experimental conditions. Using a *C. elegans* whole genome DNA microarray in this study, the effects of five different xenobiotics on the gene expression of the nematode were investigated. The exposure time for the following five applied compounds β-NF (5 mg/l), Fla (0.5 mg/l), atrazine (25 mg/l), clofibrate (10 mg/l) and DES (0.5 mg/l) was 48 ± 5 h. The analysis of the data showed a clear induction of 203 genes belonging to different families like the cytochromes P450, UDP-glucoronosyltransferases (UDPGT), glutathione *S*-transferases (GST), carboxylesterases, collagenes, C-type lectins and others. Under the applied conditions, fluoranthene was able to induce most of the induceable genes, followed by clofibrate, atrazine, β-naphthoflavone and diethylstilbestrol. A decreased expression could be shown for 153 genes with atrazine having the strongest effect followed by fluoranthene, diethylstilbestrol, β-naphthoflavone and clofibrate. For upregulated genes a change ranging from approximately 2.1- till 42.3-fold and for downregulated genes from approximately 2.1 till 6.6-fold of gene expression could be affected through the applied xenobiotics. The results confirm the applicability of the gene expression for the development of an ecotoxicological test system. Compared to classical tests the main advantages of this new approach will be the increased sensitivity and it's potential for a substance class specific effect determination as well as the large numbers of genes that can be screened rapidly at the same time and the selection of well regulated marker genes to study more in detail.


*Keywords:* *C. elegans*; Gene expression; DNA microarray; Xenobiotics

## 1. Introduction

For ecotoxicological risk assessment it is of importance to know at which concentration a contaminant becomes a pollutant, implying a toxic effect. At higher

* Corresponding author.
*E-mail addresses:* kerstinspost@web.de (K. Reichert), ramenzel@igb-berlin.de (R. Menzel).

0045-6535/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2005.01.077

environmental concentrations decreased reproduction and increased mortality are successfully used to define toxicity. But what about lower concentrated contaminants? In these cases often no significant effects could be found using classical test endpoints. The aim of this study is to investigate whether the xenobiotically induced gene expression of *Caenorhabditis elegans* could be a suitable tool to develop a screening system for environmental pollution. This new approach has the potential of a substance class specific response and should be more sensitive than classical ecotoxicological tests.

*C. elegans* is a member of the Rhabditidae, a large and diverse group of nematodes found in terrestrial habitats. Living in the pore water of the soil, the nematode is in direct contact with the dissolved pollutants. Due to its size, it can be handled like a microorganism, while offering the features of a multicellular animal. Its genome has been completely sequenced (The *C. elegans* Sequencing Consortium, 1998). Thus *C. elegans* provides the researcher with the ideal compromise between complexity and easy handling.

Since the late 1980s the nematode has been frequently used in ecotoxicological studies. The range of these studies covers reproduction, growth, movement and feeding tests (Van Kessel et al., 1988; Donkin and Dusenbery, 1993; Hitchcock et al., 1997; Traunspurger et al., 1997; Anderson et al., 2001; Höss et al., 2001), as well as the construction of transgenic strains for the monitoring of environmental stress in living worms (Jones et al., 1996; Mutwakil et al., 1997; David et al., 2003). Here the increased *hsp-16* gene expression, inducible by different kinds of stress, demonstrated for the first time in *C. elegans* the potential of the gene expression response for ecotoxicological studies.

More recently the DNA array technique has been used to investigate the whole genomic gene expression of *C. elegans* (for an overview see Kim et al., 2001). Important examples are the investigations of differentially expressed genes during development (Jiang et al., 2001) and aging (Lund et al., 2002), as well as the profiling of germline (Reinke et al., 2000) and dauer gene expression (Wang and Kim, 2003). All these studies confirmed the excellent reliability of the *C. elegans* whole genome DNA microarray created in Stuart Kim's laboratory (Stanford University, USA).

The aim of this study was to investigate the suitability of this microarray for a toxicological approach. Five different xenobiotics representing a broad range of substance classes (the herbicide atrazine, the PAHs β-naphthoflavone and fluoranthene, the drug clofibrate and the endocrine active substance diethylstilbestrol) were used to obtain information about differentially expressed genes and to draw a gene expression profile in dependence of the tested xenobiotic compound. Particularly interesting were gene families from which it is already known that members take part in phase I and II of the biotransformation system in vertebrates, e.g. the cytochrome P450 genes (CYP), UDP-glucoronosyltransferases (UDPGT), glutathione *S*-transferases (GST) or carboxylesterases (Satoh and Hosokawa, 1998; King et al., 2000; Sheweita, 2000; Bartosiewicz et al., 2001; Board et al., 2001; Schuetz, 2001; McClain et al., 2002). These genes have been particularly implicated in the biotransformation of many drugs and xenobiotics. The genome of *C. elegans* contains 80 cytochrome P450 genes, 23 UDPGT genes, about 36 GST and 17 carboxylesterases genes (data available using the euGene database: http://iubio.bio.indiana.edu:8089/worm/).

## 2. Materials and methods

### 2.1. Strain and cultivation

In this study the wild-type *C. elegans* Bristol strain (N2) was used. The nematodes were cultured in liquid medium at 20 °C as described by Brenner (1974) and Sulston and Hodgkin (1988), using freeze-dried *Escherichia coli* OP50 as food source. The cultures were synchronized by treatment with sodium hypochlorite (Emmons et al., 1979). Each culture was started with around 3 million nematodes in the L1 larvae-stage. Worms were harvested in the young adult stage, 48 ± 5 h after induction with the xenobiotic substance.

### 2.2. Treatment

The different xenobiotics, dissolved in DMSO, were added to the medium 24 h after starting the culture. The applied concentration was the calculated EC10 value for inhibition of reproduction (Menzel et al., 2001). The following five compounds were applied: β-NF (5 mg/l), Fla (0.5 mg/l), atrazine (25 mg/l), clofibrate (10 mg/l) and DES (0.5 mg/l). The final concentration of DMSO in the medium of the controls and the samples was 0.5%, v/v.

### 2.3. RNA preparation

The cell breakage was performed by homogenization with glass beads, subsequently total RNA was extracted using a RNeasy kit (Qiagen). To improve the results of the hybridization on the microarray, the method was changed and total RNA was extracted using Trizol (Invitrogen) (Chomczynski and Sacchi, 1987) resulting in higher yields of better quality RNA. Poly(A)$^+$ RNA was purified using an Oligotex mRNA kit (Qiagen).

### 2.4. C. elegans whole genome DNA microarray

The cDNA synthesis from the different poly(A)$^+$ RNA samples as well as the production and hybridiza-