

Abstract

Survival of earthworms in the environment depends on their ability to recognize and eliminate potential pathogens. Two closely related earthworm species *Eisenia andrei* and *Eisenia fetida* inhabit different environment with specific microbiota. Both species can be reliably determined using of species-specific primers for cytochrome c oxidase I (COI) and stringent PCR conditions.

Whereas, we did not observed any substantial differences in the expression and activity of CCF and lysozyme upon microbial challenge, the expression as well as the hemolytic activity of fetidin/lysenins was considerably higher in *E. andrei* as compared to *E. fetida*. Genomic DNA analyses revealed significantly higher level of fetidin/lysenins in *E. andrei* compared to *E. fetida* suggesting hypothetical gene duplication.

Earthworms live in permanent close contact with microbial environment. Coelom cavity as well as the gut of *E. andrei* earthworm differs in the number of bacteria. The number of bacteria in the gut is more than six time higher than in coelomic fluid. High microbial load of *E. coli* O55, *B. subtilis* W23, and *S. cerevisiae* S288 in the earthworm environment, resulted in an increase of microorganisms in both, the coelom and the gut. The changes in mRNA levels of defense molecules (pattern recognition receptors CCF, *EaTLR*, *EaLBP/BPI* and antimicrobial molecules lysozyme and fetidin/lysenins) in the coelomocytes and gut tissue were determined by quantitative PCR. The immune response at a cellular level was determined in histological sections, and the expression of CCF and *EaLBP/BPI* was localized using *in situ* hybridization. The immune response in gut tissue is less affected by microbial stimulation because the epithelial cells of the gut exhibit basically high mRNA synthesis of *ccf* and *eatlr* as a defense against the continuous microbial load in the gut lumen. This defense is also supported by variability and number of TLRs in the gut and increased gut enzyme activities as protease, laminarinase, and glucosaminidase, which are important for the release and recognition of molecular patterns by pattern-recognition molecules.

The cellular immune response in the coelomic cavity is mediated by coelomocytes released from the mesenchymal lining. Coelomocytes respond to the presence of bacteria by increasing the mRNA levels of pattern recognition receptors, especially CCF, *EaLBP/BPI*, *EaTLR* and an important iron storage molecule ferritin.

EaLBP/BPI as one of the LPS-binding molecules is constitutively expressed in coelomocytes and seminal vesicles.

The exposition of *E. andrei* earthworms to dioxin-contaminated soil caused damage of intestinal wall and adjacent chloragogenous tissue. It was also shown high gene expression of oxidative stress molecules calreticulin (CRT), Hsp70 and defense molecule CCF. But higher expression of CCF was probably caused by the effect of microbial biomass than the pollutant itself.

These results indicate that immune-related molecules can be useful for monitoring of soil contamination but the microbiota cannot be overlooked in the evaluation of the results.