Antimicrobial peptides in *Eisenia andrei* earthworms: their role in immune response, regeneration process and interactions with metal nanoparticles

PhD Thesis

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1. Summary

Eisenia earthworms are widely applied experimental animals in toxicological, comparative immunological and developmental biological studies. In my thesis, I concentrated on these scientific areas mainly focusing on the evolutionarily conserved immunobiological processes applying *Eisenia spp.* earthworms as an invertebrate model organism.

In the first part of my studies, we identified two mRNA homologues of the lumbricin (antimicrobial protein) *in E. andrei* that was initially described in *Lumbricus rubellus* earthworms. These novel lumbricin homologues have strong sequence identity compared to the lumbricins described from other annelids. At the tissue level, their highest mRNA expression patterns were detected in the proximal part of the intestine. In opposite to preliminary studies, both novel lumbricin homologous were detectable in coelomocytes and throughout earthworm's embryogenesis. We found that they are inducible after 48 hours of Gram-positive bacterial treatment *in vivo*. Our results support the strong conservation of lumbricin proteins within the *Annelida* group and their crucial role in the maintenance of homeostasis.

In the second part of the thesis, we examined the immunobiological processes besides the morphological changes during anterior and posterior segment regeneration of *E. andrei*. According to our results, coelomocytes migrate to the newly formed segments and play an important role in successful regeneration. Genes involved in the immune responses (pattern recognition receptors and antimicrobial proteins) predominantly indicated diminished mRNA expression patterns compared to intact worms. During the anterior and posterior restoration process, we noticed several characteristic changes at both cellular and molecular levels of earthworm's immune response, which can be compared with the immunological process explored in the regeneration of other invertebrates.

In the third part, we studied the sensitivity of coelomocytes isolated from two closely-related earthworm species (*E. andrei* and *E. fetida*) following silver (Ag) and gold (Au) nanoparticle (NPs) exposures *in vitro*. According to our results, AgNPs have detrimental effects at low concentrations and apoptosis-inducing properties towards coelomocytes that was not observed by AuNPs. Furthermore, differences were established between species in gene expression levels and in the biomolecular corona composition around the NPs, which may explain the variations in sensitivity. Our outcomes may shed more light on how the nanomaterials can affect the immune system of higher organisms.

2. General introduction

Earthworms (classis: *Clitellata*, subclassis: *Oligochaeta*) are primarily applied model organisms in toxicological research. Their experimental use can be explained by the fact that they have high heavy metal resistance and are capable of temporarily storing as well as inactivating heavy metal ions.

The first, passive layer of protection is the body wall, which separates the animal's body from the outer environment. It is composed of epidermis and muscle layers. Besides it consists of a thin layer of mucopolysaccharide containing cuticle, it also encompasses proteins, thereby acting as an antimicrobial barrier[1].

During evolution, earthworms have developed efficient immune mechanisms against environmental pathogens. In addition, here appeared first the co-operation between cellular and humoral immune components. Humoral immune processes are attributed to the coelomic fluid in the coelomic cavity. It is known that earthworm's coelomic fluid possesses a wide range of biological functions including proteolytic, hemolytic, antimicrobial and cytotoxic activities[2]. Earthworm's coelomocytes (macrophage-like cells) are part of the cellular immune response. These cells are both morphologically and functionally equivalent to vertebrate leukocytes[3].

Subpopulations of coelomocytes have many different functions; the most important ones are the phagocytosis, encapsulation and cellular cytotoxicity. Coelomocytes were initially characterized by classical light and electron microscopy-based techniques. Recently, by a simpler classification -based on flow cytometry and application of specific mAbs- coelomocytes can be further distributed into two major subpopulations. These subgroups are named as amoebocytes (hyaline and granular) and eleocytes[3,4]. According to our previous results, hyaline and granular amoebocytes are capable of phagocytosis and encapsulation and these effector cells also express various pattern recognition receptors (PRRs). Chloragocytes/eleocytes have a detoxifying role through their lysosomal system. These cells are attributed to the synthesis of hemoglobin, the maintenance of pH balance and isohydria. More importantly, eleocytes produce several bioactive proteins (e.g.lysenin) that are involved in humoral immune responses[2,3].

Coelomocytes are considered to be essential constituents because of their important role in the innate immune response. They resemble macrophages due to their diverse functions and role in phagocytosis. Furthermore, they are able to participate in antimicrobial peptide secretion and lytic reactions against target cells.

3. Objectives

Earthworms are widely used model animals in comparative immunology, developmental and regenerative biology as well as in toxicological research. In my thesis I present here three separate chapters, where these aforementioned areas are highlighted with partly or completely new aims. Indeed, in all chapters, I focus on the evolutionarily conserved immunobiological processes.

1. Initially, lumbricin -which is proline-rich antimicrobial peptide- has been isolated and characterized from the earthworm, *Lumbricus rubellus*. By now, several lumbricin homologues have been identified and described from other earthworm and leech species (e.g. *Hirudo medicinalis*). Due to the close relationship, we hypothesized the presence of lumbricin in *Eisenia andrei* earthworms. Therefore, **the purposes of the study** are to isolate **the potential homologue of lumbricin in *E. andrei*,** assess the **phylogenetic relationships** and characterize **its mRNA expression pattern in different tissues and organs** of adult earthworms as well as **throughout earthworm's ontogenesis.** Furthermore, we aimed to examine its mRNA expression pattern upon **pathogen (bacteria, fungi) exposures *in vivo***.

2. Regeneration is a morphologically well-studied process, but only limited knowledge is accessible about the molecular interactions of restoration and immune-related mechanisms. Indeed, *E. andrei* earthworms have an extensive regeneration capacity; however, even the molecular aspects of embryonic development (e.g. cell division and cell death) have not been explored yet in details. Therefore, **our principal aims were to observe the cell proliferation and cell death in the course of segment regeneration. In addition, we monitored the occurrence of cellular immune components and the mRNA expression patterns of humoral immune factors** (e.g. *lumbricin, lysenin)* during the regeneration kinetics.

3. As for toxicological point of view, **we investigated the sensitivity differences of coelomocyte from two closely-related *Eisenia* species (*E. andrei* and *E. fetida*) at different biological levels after noble metal (Ag and Au) nanomaterial treatments.** Upon lethal concentrations, **we aimed to detect oxidative stress, apoptotic response, and DNA-damage.** Sublethal concentrations were applied **to untangle the gene,- protein secretion and protein corona composition differences of the two species.**

4. Identification of novel lumbricin homologues in *Eisenia andrei* earthworms

4.1. Introduction

Antimicrobial peptides (AMPs) are biologically active and multifunctional effector molecules that are structurally conserved during phylogenesis. Due to their cationic and positively charged structure, they possess a broad range of antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, protozoa and enveloped viruses. Previous studies have revealed that their antimicrobial activity greatly depends on their amino acid composition and physical-chemical features[5].

Invertebrate organisms like *E. andrei* earthworms operate with distinct innate cellular and humoral immune components to maintain their self-integrity. Lumbricin was firstly identified in the earthworm, *Lumbricus rubellus*[6]. By now, several lumbricin homologues have been described from other earthworm and leech species (*Metaphire tschilliensis*, *M. guillelmi, Hirudomedicinalis*). Recent evidence suggests that this molecule exhibits *in vitro* a broad range of antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi without haemolytic activity. The study aimed to isolate the potential homologue of lumbricin from *E. andrei* earthworm and characterize its expression pattern in diverse tissues and organs of adult earthworms, during embryonic development and upon *in vivo* pathogen challenge.

4.2. Materials and methods

4.2.1. Earthworm husbandry, RNA isolation, cDNA synthesis and rapid amplification of cDNAends (RACE)

4.2.2. Sequence and phylogenetic analysis

4.2.3. Isolation and sorting of coelomocytes, flow cytometry, cell sorting

4.2.4. RNA isolation, cDNA synthesis and semi-quantitative PCR

4.2.5. Relative quantification of target genes by qPCR from adult tissues and embryos

4.2.6. *In vivo* bacterial and zymosan treatments, statistical analysis

4.3. Results

Based on the available annelid lumbricin sequences a novel generic forward primer was designed for the detection of lumbricin homologues in *E. andrei* by 3'RACE PCR. Surprisingly, the 3'RACE PCR showed the presence of not one but two discrete bands. The sequences determined from the two PCR amplicons showed only 43% pairwise nucleotide (nt) identity. Using sequence-specific primers and 5' RACE PCR technique 466-nt and 549-nt-long sequences (without the polyA-tail) of the two mRNAs were determined. The 466-nt-long mRNA called as lumbricin (Lumbr) codes a 63-aa-long peptide (average calculated molecular mass: 7413.35 Da. On the other hand, the 575-nt-long mRNA called lumbricin-related peptide (LuRP) encodes a 59-aa-long peptide (average calculated molecular mass: 7066.84 Da.

The phylogenetic ramifications revealed a strong sequence identity among annelids. The precursor peptides showed 98% (Lumbr) and 66% (LuRP) identity to the antimicrobial peptide lumbricin-1 from *L. rubellus* (AF06552), however, the *E. andrei* Lumbr (KX816866) and *E. andrei* LuRP (KX816867) precursor peptides demonstrated only 66% pairwise aa identity. Despite the strong sequence identity between Lumbr and LuRP, the precursor peptides are separated from each other. Lumbr is clustered together with the lumbricin-1 of *L. rubellus*, while LuRP shows a closer relationship to the lumbricin homologue from *H. medicinalis.* In general typical lengths of lumbricin homologues are ranged between 57 and 76 amino acids. Additionally, the prominent property of these AMP family proteins is their high proline content. Furthermore, their aromatic amino acid (His, Trp, Tyr) content is relatively high (15–16% in molar ratio), which could further suggest the antimicrobial activity of these *Eisenia* lumbricin homologue peptides.

The highest mRNA expressions of both AMPs were detected in the proximal part of the intestine (including pharynx and gizzard), while other tested tissues had a moderate (body wall, midgut, ovary, seminal vesicle, metanephridium, ventral nerve cord) or low (coelomocytes) level of expression. Higher *LuRP* mRNA expression was demonstrated in all tested tissues and coelomocytes compared to *Lumbr*. The highest expressions of both AMPs were detected in the intestine because this organ is the most exposed for frequent microbial invasions. Only the amoebocyte subpopulation was positive for *Lumbr* and *LuRP* expression as opposed to the eleocyte subpopulation.

We observed that both lumbricin homologues from *E. andrei* were expressed in the course of embryonic development. Their expression displayed continuous increase up to the

fourth developmental stage (E4) when the body is entirely segmented and the organ differentiation is completed. *LuRP* exhibited significantly higher expression compared to *Lumbr* in the different stages of developing *E. andrei* earthworms.

Our results evidenced a rather slow induction in genes of tested upon pathogen challenge. Specifically, both *Lumbr* and *LuRP* mRNA levels became significantly elevated upon the 48 h of *Staphylococcus aureus* bacteria challenge, but there was no any increase of expression upon *Escherichia coli*or zymosan treatments.

4.4. Summary

Earthworms possess refined innate immune mechanisms against environmental pathogens and during evolution firstly here became apparent the close interactions between cellular and humoral immune compartments. The production of AMPs, which are the humoral elements of the innate immune system, can be observed from prokaryotes to higher vertebrates (e.g. mammals). However, in the case of earthworms, data on AMPs are rather limited.

Our study has revealed the presence of two novel members from the proline-rich lumbricin AMP family in the earthworm *E. andrei* as well as their high conservation among lumbricin homologues[7,8,9]. Strong expression of *Lumbr* and *LuRP* was observed in several organs and tissues. Differential expression of immune molecules during earthworm's embryogenesis are rather unexplored, therefore it can be of interest in the future studies. Based on our research outcomes, the slow induction of *Lumbr* and *LuRP* (after 48 hours) was perceived by *S. auresus* treatment *in vivo*.

Our novel data support the high conservation of lumbricin AMPs in annelid worms and their possible role in the maintenance of earthworm immune homeostasis during ontogeny and pathogenic infections.

5. Role of the cellular and humoral immune components in tissue regeneration of *Eisenia andrei*

5.1. Introduction

Regeneration is a series of morphogenetic events, where the injured organ or tissue is partially or completely restored[10].

In recent years, several steps have been attempted to understand the molecular aspects of regeneration. Among invertebrates, most annelids are able to regenerate their lost body

parts. Their regeneration is accomplished by the combination of epimorphic recovery (forming of blastema with progenitor cells) and morphallaxis (re-organization of intact segments) [11]. During evolution, the extensive earthworm regeneration capacity has probably developed to deal with predators[10]. Regeneration is well-studied morphologically, but the molecular background in *Oligochaeta* earthworms is less understood. Most molecular studies have been mainly focused on the expression of specific genes that were already known to play a role in development. In contrast our knowledge is limited to the relationships of regeneration and immunological processes.

To date, numerous studies have shown that the immune system plays a crucial role in the successful regeneration process. More specifically, it seems that the regenerative capacity is inversely proportional with the activity of the immune system. Our principal aims were to determine the cell proliferation and cell death during segment restoration. In addition, the involvement of coelomocytes and immune-related genes expression were monitored in the course of 4 weeks anterior and posterior regeneration process in *E. andrei*.

5.2. Materials and methods

5.2.1. Earthworms husbandry and execution of regeneration experiments

5.2.2. Histochemisty and enzyme histochemistry

5.2.3. Assays for cell proliferation and death, immunofluorescence analysis

5.2.4. RNA-isolation, reverse transcription and real-time PCR

5.2.5. Statistical analysis

5.3. Results

We performed standard histochemical stainings to evaluate the initiation of blastema (4^{th} day) to the end of observation period (4^{th} week of regeneration). Based on the hematoxylin-eosin staining new segments and tissue remodeling were observed in both anterior and posterior regeneration. PAS staining for carbohydrate/glycogen have not evidenced remarkable differences between the intact and regenerating earthworms. In contrast, acid-phosphatase staining (a marker of lysosomal activity) marked significantly stronger reaction between the anterior and posterior blastemas vs intact segments.

Notable variations were found in the number of dividing cells among intact animals and 2/4-week-old anterior and posterior blastema. Interestingly, cell division is much more pronounced during anterior rather than the posterior regeneration process.

Both eleocytes and granular amoebocytes migrated into the newly formed segments. However, much less anti-EFCC5-positive eleocytes and anti-EFCC4-positive granular amoebocytes were observed during the 2 and 4 weeks anterior regeneration compared to the posterior counterparts. Contrastingly, anti-EFCC5 positive eleocytes were apparent and formed clusters in the lacuna of the 2nd week regenerative blastema. The presence of lysenin-secreting eleocytes and thus the formation of an antimicrobial environment during the regeneration process may be associated with protection against pathogens. Apoptosis was detectable during the entire regeneration process. Most pronounced level of cell death was observed at the 4th week of anterior and posterior regenerating animals. Granular amoebocytes were visible in the close vicinity of apoptotic cells, so probably they are involved in the clearance mechanism. Anti-EFCC4-positive granular amoebocytes are capable of migration and phagocytosis. They were also noticeable in the newly formed blastema not only in the coelomic cavity but in muscle layers as well.

Throughout the regeneration period, mRNA expression of immune response-related genes was reduced compared to intact animals. In the anterior and posterior blastema, *toll-like receptor* showed similar levels, but biased expression of some AMPs (*lysenin*, *lumbricin*) were observed. While cell division was more pronounced (2nd week), both *Lumbr* and *LuRP* mRNA expression was very low, but after proliferation was significantly reduced and the aforementioned AMPs mRNA expression started to increase.

5.2.4. Summary

Regenerative ability is an ancient trait and its success is determined by certain factors[10]. Several characteristic cellular and molecular alterations have been observed in the innate immune responses during the anterior and posterior regeneration of *E. andrei* earthworms.

Relying on our results there are morphological differences during anterior and posterior regeneration. Coelomocytes are involved in the regeneration process, but immune response-related mRNA expression (PRR, AMPs) has attenuated expression compared to intact animals. So far, corresponding studies (including in earthworms and higher organisms) have focused mainly on the expression of genes required in development[12], because today it is thought that regeneration can be interpreted as an alternative way of embryonic development.

This immunological unresponsiveness phenomenon has been already described during the regeneration of other invertebrate or vertebrate species. Down-regulation of immune-related genes may indicate an immature characteristic of blastema and most probably the metabolism may be also biased compared to the differentiated tissues. By another theory, down-regulation of immune-related genes can be explicable with distinct allocation strategies and life-history "trade-offs" between regeneration and immune functions. Thus, the blastema might temporarily be an immunologically tolerated area, which is likely to the reason for successful regeneration[13,14].

6. Evolutionarily conserved stress and immunotoxicological processes: *in vitro* interactions of silver and gold nanoparticles with invertebrate immune cells

6.1. Introduction

Nanotoxicology is considered to be a novel ramification of toxicology that investigating the interactions and potential adverse effects of nanoparticles on living organisms. Our knowledge is rather limited to the effects of NPs on the immune system of living organisms. Nano-sized materials are more susceptible to chemical reactions and they can be more mobile than similar materials of larger sizes due to their different strengths or electrical properties[15].

Silver (Ag) NPs are the most widely used NPs in commercial products owing to their antimicrobial properties, while gold (Au) NPs are mainly utilized in medical diagnostic imaging. Numerous studies have reported the cytotoxic effects of NPs (mainly AgNPs). It is confirmed that smaller NPs are able to induce a greater level of ROS generation leading to an increased apoptotic response. Furthermore, several studies have shown a strong correlation among elevated oxidative stress, mitochondrial membrane and DNA damage[16]. In most cases, AuNP exposure has no effect on cell viability and the outcome is still unrevealed. Besides, AgNPs and AuNPs are capable of interact with proteins in biological fluids (e.g. cell culture supernatant, serum) resulting in the formation of a bio-molecular corona (so-called protein corona) [17].

Eisenia andrei and *E. fetida* are two distinct but closely-related earthworm species by their minor morphological features, which are widely used in standardized toxicological studies[18].

In this work, we observed the biological interactions between earthworm immune cells and 10 nm noble metal NPs (AgNPs and AuNPs) at diverse biological levels *in vitro*. Our

goals were to determine the molecular and cellular response towards NPs, to study the role of the antimicrobial molecules (lysenin, lumbricin, LuRP) and to determine the sensitivity of coelomocytes from the two species.

6.2. Methods

6.2.1. Extrusion of coelomocytes and *in vitro* exposure conditions

6.2.2. Concentration-response curve fitting, and the choice of test concentrations

6.2.3. Gene expression profiling

6.2.4. *Ex-situ* protein corona formation and protein secretion studies

6.2.5. Statistical analysis

6.3. Results

In both earthworm species, AgNP (and $AgNO_3$ at lower concentrations) but not AuNPs exerted concentration-dependent toxicity against coelomocytes within the concentration range tested. As for the species differences, coelomocytes from *E. fetida* showed higher sensitivity towards $AgNO_3$ and AgNPs than *E. andrei* coelomocytes based on the estimated lower LC_x values and the steeper Hill-Slopes. For gene and protein expression experiments, we opted for low-cytotoxic concentrations (LC_{20}) determined for $AgNO_3$ and AgNPs and, while AuNPs were applied at the same concentration.

Overall gene expression patterns were not largely different between the two species, but according to the relative gene expression profiles *E. fetida* coelomocytes were more sensitive to the studied treatments. The expression profiles were exactly opposite at 2 h, more specifically *E. andrei* had low *metallothionein* (*MT)*/high *lysenin* profile and *E. fetida* had a high *MT*/low *lysenin* profile. Most striking was that AgNPs -typically for both species- provoked a gradual down-regulation in *lysenin* towards 24 h at the same time with the induction of *superoxide dismutase* (*SOD*) and in *E. fetida* with alike *MT* (*Lumbr* and *LuRP* expressions were variable between species).

As for the species differences, we have noted a clear distinction in lysenin proteins (38 kDa and 40 kDa bands) between *E. andrei* and *E. fetida* coelomic proteins and thus the resulting protein coronas around AgNPs.In addition to the lysenin protein family, actin is also a constituent of protein coronas formed around AgNPs.

For both earthworm species, exposure to AgNPs or AuNPs initially resulted in higher secretion of lysenins at 4 h compared to the controls. Subsequently, the amount of lysenins diminished at 24 h concurrently with down-regulation of the gene expression.

6.4. Conclusions

In the present study the differential sensitivity of coelomocytes from two closely-related earthworm species were demonstrated towards noble metal NPs. In general, *E. fetida* coelomocytes showed greater sensitivity to AgNPs compared to *E. andrei* coelomocytes, whereas we could not determine species sensitivity to AuNPs for the concentration range tested.

The gene expression profiles indeed suggest the involvement of antioxidant mechanisms such as *SOD* in both species, and persistent up-regulation of *MT* in *E. fetida* underscoring the thiol-mediated detoxification process towards 24 h[19]. In both species, expression/secretion of lysenins seems to be stress-regulated and this implies a complex feedback mechanism for AgNPs because lysenins are specifically enriched by AgNPs[17,19]. There are also differences in the basal expression levels between species, accurately *lysenin*, *lumbricin*, *SOD* showed higher expression in *E. andrei*, whereas, in *E. fetida LuRP*, *toll-like receptor* (*TLR*) and *MT* genes are strongly inducible under stress conditions. Even though AuNPs did not result in cell death, but it caused biased the gene expression[20]. Therefore the potential impact of AuNPs on innate immunity may deserve further attention.

One possible explanation for the higher responsiveness of *E. fetida* is that its natural living environment is considerably different from that of *E. andrei*[18]. Specifically, *E. andrei* flourishes in microbe-rich compost while *E. fetida* subsists in moist forest soil, underlining genetic alterations in sensibility, susceptibility, as well as tolerance of their immune system evolved through natural selection. Furthermore, our results confirmed early (2 h) induction of *TLR* in *E. fetida*, which may be related to the prompt uptake of AgNPs into the target cells[19].

7. Summary of the new results

Eisenia earthworm species are primarily used in toxicological experiments as model organisms but it is also emerging in comparative immunological and developmental biological studies. Applying a simpler invertebrate model organism may bring us responses to unanswered questions in vertebrates and understand better certain immunological mechanisms.

1. We identified and characterized the homologue of lumbricin and its closely-related relative (LuRP) in *E. andrei*. Both AMPs demonstrated a strong degree of sequence homology to other annelid lumbricins and belong to the proline-rich AMPs family.

2. In contrast to previous observations, both AMPs indicated rising mRNA expression patterns during ontogenesis. Furthermore, the presence of these molecules has been identified in several tissues, including coelomocytes.

3. *Lumbr* and *LuRP* AMPs were inducible after 48 hrs *S. aureus* treatment *in vivo*.

4. Morphological changes were detected during anterior and posterior regeneration in *E. andrei*. The relationship between cell proliferation and programmed cell death has been evaluated along with the immunobiological mechanisms involved in the restoration process.

5. Most extensive cell proliferation was perceived after two weeks, while apoptosis was detected throughout the regeneration process, more intensively during the wound healing and tissue remodeling phase.

6. Cellular elements of the innate immune system (coelomocytes) participate in the regeneration process and migrate to the blastema.

7. Immune response genes have reduced mRNA expression during anterior and posterior regeneration.

8. Toxic effects of AgNPs and AuNPs were investigated *in vitro* on coelomocytes of two closely related species (*E. andrei* and *E. fetida*). AgNP induced dose-dependent cell death in the coelomocytes. *E. fetida* coelomocytes evidenced greater sensitivity than *E. andrei* coelomocytes.

9. The overall gene expression pattern was not significantly different between the two species. At the 24 hour treatment, *lumbricin* expression was also decreased in *E. andrei* and *E. fetida*, in contrast, *LuRP* became elevated.

10. After 2 hours of AgNP and $AgNO_3$ treatments, *E. andrei* had a low *MT*/high *lysenin* profile and *E. fetida* had a high *MT*/low *lysenin* profile. The suppression of *lysenin* and

induction of *SOD* was apparent by the end of 24 hrs AgNP treatment in *E. fetida* coelomocytes.

11. The enrichment of coelomic proteins (CPs) (bands 38, 40 and 45 kDa) was observed in protein coronas formed around AgNPs and AuNPs.

12. Western blot and LC-MS/MS analysis reinforced that lysenin binding is only restricted to AgNPs. In the case of *E. fetida* CPs both lysenin isoforms were attributed in protein corona to AgNPs.

13. In addition to lysenin proteins, actin may play an indispensable role in the protein corona formation.

14. After 4 hours, the lysenin secretion profile was consistent with the gene expression kinetics in both species. High levels of lysenin (both lysenin isoforms in *E. fetida*) were detected by AgNP and AuNP treatments, which showed a significant decrease by the end of the 24 hour treatment.

15. Low concentrations of AgNP and AuNP treatments have strongly influenced the lysenin expression among the tested earthworm antimicrobial molecules.

8. References

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10. Publication list

Publications related to the thesis

Engelmann P., Hayashi Y., **Bodó K.**, Ernszt D., Somogyi I., Steib A., Orbán J., Pollák E., Nyitrai M., Németh P., Molnár L. (2016a). Phenotypic and functional characterization of earthworm coelomocyte subsets: Linking light scatter-based cell typing and imaging of the sorted populations. *Dev. Comp. Immunol*. **65**, 41-52. *Independent citations: 7, IF: 3,218.*

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TDK presentations

PTE TTK Tudományos Diákköri Konferencia Biológia Szekció Pécs, 2016. Fém nanopartikulumok és *Eisenia* coelomasejtek kölcsönhatásának vizsgálata: *in vitro* tanulmányok.

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