



Original Research Article

Antimicrobial activity of Earthworm Coelomic fluid against disease causing microorganisms

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A B S T R A C T

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Earthworms are the first annulated eucoelomate organism with complete digestive system, closed circulatory system with haemoglobin in the plasma as carrier of oxygen and carbon di oxide. The antimicrobial activity of earthworm coelomic fluid *Lampito mauritii* and *Megascolex konkanensis*, *Drawida impertusa* and *Drawida lennora* were confined against disease causing microorganisms through the inhibition zone formation. The coelomic fluid of earthworms *Lampito mauritii* and *Megascolex konkanensis* have high antimicrobial activity when compare to *Drawida impertusa* and *Drawida lennora* against *Aeromonas hydrophila*, *Bacillus subtilus*, *Vibrio para haemolyticus*.

Introduction

Earthworms belong to the phylum Annelida class oligochaeta and evolved in last Precambrian period (Darwin 1890). Earthworms are nocturnal soft-bodied saprotropic invertebrates of agro ecosystem and also one of the major macro fauna of the soil biota. They occupy a very unique position in the Animal kingdom and that have successfully invaded the terrestrial habitats. The body of earthworm is divided into a series of uniformly placed segments (annuli). This nature of division of the body both externally and internally has enabled animal to have flexibility and for the initiation of development of good musculature. Earthworms are the first group of animals to have complete digestive system, closed circulatory system with

haemoglobin in the plasma as carrier of oxygen and carbon di oxide.

With the development of biotechnology, a bioactive compound in earthworm has already caused the attention of more and more scientists (Yan Shu Fang et al 1999., Cooper 2002., Yu Shen 2010). This is because the medicinal value of earthworm is related to many chemical components. Researchers have investigated some features and activities of some enzymes isolated, purified and even expressed many components.

Infection by a pathogen represents one of the major threats to any living organism. These pathogens are firstly bacteria living in

water or soil that are ingested during feeding or introduced into the body following injury. Parasites, particularly larval forms, which represent the dissemination phase, are another important group of potentially pathogenic agents. During the course of evolution, earthworms have developed defense strategies against these living pathogens. Earthworms lack true antibodies and hence an adaptive immune response and instead have efficient innate immunity system to defend themselves against invading foreign materials. In living organisms, peptides are an important defense component, many peptides were found in various living organisms. Therefore it can be supposed that earthworm living in the pathogen-abundant environment must have peptides against bacteria. In earthworms innate immunity is maintained by cellular components, different coelomocytes (leukocytes), housed in coelomic cavity whose fluid also contains many immunological (antimicrobial) active molecules.

Vast literature exists on various aspects of ecology, physiology and behavior of earthworm and their functional role in certain ecosystems, especially in the temperate and tropical regions of the world. On the contrary, very little work has been done on the medicinal aspects (Reynolds and Reynolds 1972, 1979).

Materials and Methods

Earthworms were collected from the soil of different habitat by digging and hand sorting method. The native species of earthworms *Lampito mauritii*, *Megascolex konkanensis*, *Drawida impertusa* and *Drawida lennora*, were identified. Coelomic fluid is obtained by actual washing of earthworms. Earthworms release the coelomic fluid along with mucus through the pores present on the

dorsal surface of their body called dorsal pores. This is a natural process to keep their body surface moist that acts as respiratory organ. As the pores remain open all the time, slight stimulation by either cold or hot shock makes them to release the coelomic fluid

Coelomic fluid can be directly collected from the body cavity of earthworms without causing any harm to them. In this method of collecting the fluid, three to four earthworms are taken in an approximately 10cm diameter Petri plate and holding the plates in a slanting position and keep earthworms pointing downwards. Cold shock is given to earthworm by gently moving a small beaker containing a few ice cubes. The coelomic fluid released due to cold shock drips and gets collected at the lower side of the Petri plate. This fluid can be pipette out using a sterilized pipette with fine nozzle. This is the pure coelomic fluid that can be used for different biological investigations (Kale, 2006).

The gram positive and gram negative disease causing micro organism namely *Aeromonas hydrophila*, *Bacillus subtilis*, *Vibrio para haemolyticus* were collected from Government Hospital, Erode, Tamil Nadu, India and cultured the microbes in fresh sterile nutrient agar medium and used gel puncher into the medium. Drops of collected coelomic fluid were transferred into the culture medium and all the plates were incubated in 37°C for 24 hours.

The SDS-PAGE was performed on a 10% acryl amide native gel at 4°C using common buffer system without and with SDS. For native gels, the samples were not denatured before electrophoresis. The coelomic fluid (30µl/gel) was applied to the gel and allowed to migrate for three hours. SDS-PAGE with 3% stacking gel and 10% separating gel was carried out. Coelomic

fluid were treated with an equal volume of the sample buffer containing 2% SDS, 10% glycerol with 5% 2-mercapto ethanol at 100°C for 2 min. Gels were run in a solution of 50mM Tris, 400mM glycine and 0.1% SDS at 16 mA in the stacking gel and at 24mA in the separating gel until the bromophenol blue in a solution of 40% ethanol / 10% acetic acid for one hour and destained with 20% ethanol / 5% acetic acid / 2.5% glycerol over night.

Results and Discussion

The present work is supposed to be the first extensive work in antimicrobial activity of Indian earthworm species. To test the antimicrobial activity of coelomic fluid, the inhibition zone formation around the coelomic fluid was noted and measured the

diameter of that inhibition zone range.

The antimicrobial activity of earthworm coelomic fluid *Lampito mauritii* and *Megascolex konkanensis* were confined against disease causing microorganism namely *Aeromonas hydrophila*, *Bacillus subtilis*, *Vibrio para haemolyticus* through the inhibition zone formation ranging at range 7-17 mm and 9-15 mm respectively.

The antimicrobial activity of earthworm coelomic fluid *Drawida impertusa* and *Drawida lennora* have moderate activity against disease causing microorganism namely *Aeromonas hydrophila*, *Bacillus subtilis*, *Vibrio para haemolyticus* were confined through their inhibition zone range from 3 -6 mm and 3- 8 mm respectively.

Tabel.1

Name of the species	<i>Aeromonas hydrophila</i>	<i>Vibrio para haemolyticus</i>	<i>Bacillus subtilis</i>
<i>Lampito mauritii</i>	17 mm	0.7cm	1.3cm
<i>Megascolex konkanensis</i>	15 mm	0.9cm	1.2cm
<i>Drawida impertura</i>	5 mm	0.3cm	0.6cm
<i>Drawida lennora</i>	3 mm	0.5cm	0.8cm

Collection of coelomic fluid



Lampito maritii

Control plate



Megascolex konkanensis

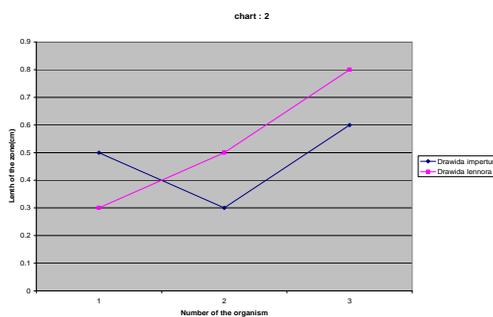
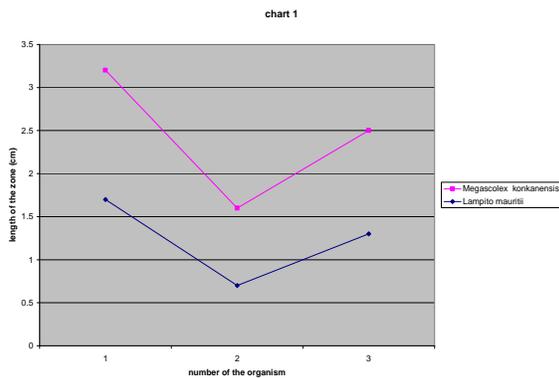


Drawida lennora

Drawida impertusa



Comparison of Zone Formation



1. *Aeromonas hydrophila* 2. *Vibrio parahaemolyticus* 3. *Bacillus subtilis*

Earthworm is a first terrestrial invaded organism and if serving on soil with million of microorganisms. The antimicrobial activity in the coelomic fluid of earthworm may be because of innate immune mechanism and detect

microorganism by recognizing conserved molecular pattern (Popovic *et al* 1996)

The Asian countries like China and Korea are aimed at using earthworms in pharmaceutical applications. They have

isolated enzymes and other active principles that can serve as antibiotics and anti-tumor agents. They have made detail studies on fibrinolytic enzymes of earthworms have also found their way in the preparation of cosmetics as a factor to delay ageing (Zen 2004). The metallothianins in earthworm body fluid is considered to bring down the toxicity level in human body caused due to pollutants in the environment. The logic behind this study was that the earthworms are found in the decomposing waste matter and to overcome effects of such adverse conditions, they must have evolved themselves a good immunity system.

Lampito mauritii and *Megascolex konkanensis* belong to the family of Megascolecidae and *Drawida impertusa* and *Drawida lennora* comes under the family Moniligastridae. Most of the *Megascolecidae* family species have melanin pigment in their body, but primitive family Moniligastridae species contains very less melanin pigment in their body. In the above study we got good antimicrobial activity in *Lampito mauritii*, and *Megascolex konkanensis*. So the antimicrobial activity was varied from species to species. The disease causing microorganism of *Aeromonas hydrophila* are gram negative rod species, its found in water, soil, food, animal and human feces. *Aeromonas hydrophila* are causes wound infections, diarrhea, especially in immuno compromised patients, and mainly affecting in fish.

Vibrio para haemolyticus were gram negative rod species. It transmitted through ingestion of raw or under cooked seafood, especially shellfish such as oysters. It's a major cause of diarrhea. Clinical picture caused by *Vibrio para haemolyticus* varies from mild to quite

severe watery diarrhea, nausea, and vomiting abdominal cramps and fever.

Here we selected the four species of earthworms coelomic fluid namely *Lampito mauritii*, *Megascolex konkanensis* and *Drawida impertusa*, *Drawida lennora* and found the antimicrobial activity against disease causing microorganism and also we confined through the presence of protein molecule using SDS-PAGE method. The effective band formation of protein molecule is present in *Lampito mauritii*, *Megascolex konkanensis* and the other two earthworm species *Drawida impertura*, *Drawida lennora* coelomic fluid having moderate protein band formation. By inoculation of bacteria into the coelomic cavity at earthworm, the coelomocytes initial the process of connecting with each other by their adhesive structures around the bacterial and form so called "brown bodies" (Valembois *et al* 1992).

Supportively study of Hrzenjak *et al* 1992 documented the coelomic cytofactor I isolated from the coelomic fluid is involved in the activation at prophenoloxidase cascade via recognition at gram negative bacterial cell wall molecules, such as glucan and lipopolysaccharied. At the present level of knowledge it is difficult to define which molecules at G-90 are responsible for its antibacterial activity. Theoretically, such activities could be some of the following molecules at molecular masses 33, 40, 42, 45, and 60KDa, which were detected by SDS-PAGE in G-90. By immunochemical analysis there proteins were shown to belong to the immunoglobulin super family (Popovic *et al.*, 1998). Considering the demonstrated biological activities at G-90, assumed that the macromolecules in the G-90 mixture are present in

biologically balanced properties and they act pleiotropically. These findings confirm the suggestions by (Cooper *et al* 2002).

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