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***Research Article***

# Isolation of Coelomic Fluid from Earthworm and its Antibaterial Activity

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**Abstract**

In naturally, earthworms were secreted the coelomic fluids which helps in the movement of earthworm and also it consist of many proteins which are responsible for microbial resistant from environment. The present work that the coelomic fluids were collected using cold shock method and were separated from debris by centrifugation method and the isolated coelomic fluid was tested the concentration of protein presence by Bradford's method and the proteins are analyzed using SDS-PAGE. Then the antimicrobial activity was done through the agar well diffusion technique with different concentrations of crude coelomic fluids. The result revealed that the antibacterial activity in earthworm shows that the zone of inhibition of coelomic fluid against *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis.* The present work conclude that the *Eudrilus eugeniae* coelomic fluid shows antibacterial properties against selected bacterial isolates and suggests some of the coelomic fluid components might be useful for pharmaceutical applications in future.

**Keywords:** Coelomic fluid; SDS-PAGE; Bradford; Anti-bacterial activity.

## Introduction

Earthworms are essential organisms in soil. They cause soil fertility through their burrowing, ingestion and excretion [1]. The earthworm is a tube-shaped, segmented worm found in the phylum Annelida. Earthworms are commonly found living in soil, feeding on live and dead organic matter [2]. An earthworm's digestive system runs through the length of its body [3]. It conducts respiration through its skin. It has a double transport system composed of coelomic fluid that moves within the fluid-filled coelom. While earthworms, the largest of the Oligochaeta, have medicinal properties, they are also related to various other species, such as leeches, that have been shown to exhibit therapeutic benefit [4]. The segmented earthworm`s body cavity is filled up with coelomic fluid.

Modern medical research has indicated that the coelomic fluid (CF) of earthworms contains an abundance of bioactive substances including lectin [5], polysaccharide [6], protease [7], antibacterial peptide [8], metalloenzyme [9], fibrinolytic enzyme [10], and so on. Earthworm proteins and peptides have exhibited various biological activities [11, 12]. Earthworm coelomic fluid contains molecules that exhibit antibacterial properties [13]. Non-diluted coelomic fluid antibacterial activity against a broad spectrum of bacteria, including *Citrobacter freundii*, *Pantoea spp.*, *Enterobacter cloacae*, *Klebsiella terrigena*, *K. pneumophila*, *Bacillus pumilus*, *B. megaterium*, *B. cereus*, *Chryseomonas luteola*, is displayed [14]. With the development of Biotechnology, a bioactive compound in Earthworm has already caused the attention of more and more scientists [15]. The present study aims to find the antibacterial properties of earthworms *Eudrilus eugeniae* coelomic fluid against selected pathogens. The bacterial strains used were *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli.* The antibacterial studies conducted with different concentration of crude coelomic fluid using well diffusion method.

## Materials and methods

### *Earthworm: Eudrilus eugeniae*

The earthworms were collected from Periyar Maniyammai University, Vallam. Thanjavur, Tamilnadu, India.

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### *Collection of coelomic fluid by cold shock method*

In cold shock method approximately 15 grams of worms are taken from the culture pit and washed with sterile distilled water. The worms are then dried on a filter paper and placed in an aluminum foil into a "cone" shape to fit into the glass funnel. The funnel is held in a burette clamp on a titration stand. The set-up is shown fig. 1. A bag of ice that fits over the funnel was placed above the worms such that the worms could feel the drop in temperature due to the ice pack above. The coelomic fluid was made to release through the dorsal pores of its body due to the drop in temperature surrounding it [16]. The fluid was collected in a clean sterile dry test tube that was fit to the end of the funnel. The collection was carried out for 30 minutes and the worms were quickly released into a separate worm–culture pit for relaxation. The collected coelomic fluid was then centrifuged at 5000rpm for 10 minutes to deposit the debris and the clear straw-colored supernatant was then filter sterilized through 0.2m syringe filter into a clean, dry, sterile microfuge in a Laminar Air flow chamber and stored under -20ºC for future research studies.

### *Determination of protein concentration*

Protein concentration was determined by the previous normal method (Bradford, 1976), and the reagent with bovine serum albumin (BSA) was defined as the standard [17].

### *Characterization of coelomic fluid proteins by SDS- PAGE method*

The sample was subjected by SDS-PAGE (12%). The glass plates were cleaned thoroughly. Spacer strips were placed on the sides and at the bottom and sealed with agarose. Separating gel mixture was added and overlaid with a layer of acetone. The gel was allowed to polymerize. Following polymerization, the acetone layer was removed and stacking gel mixture was added on the top of the separating gel. The comb was inserted between the plates and the gel was allowed to polymerize. After polymerization, the comb and spacer at the bottom was removed and the wells were washed with distilled water. The setup was placed in electrophoretic apparatus that was later filled with running buffer. The sample was denatured by mixing with loading dye and boiling for 5 min. The samples were loaded on to the wells and electrophoresed for 2-3 hr at 100 V. The gel was subjected to CBB staining [18].

### *Antibacterial activity by agar diffusion method*

The young culture of selected pathogens *Bacillus subtilis, Staphylococcus aureus, and E. coli* were prepared in nutrient broth and lawn culture of different pathogens were prepared by swabbing young culture (16-18 hrs) in Nutrient agar and waited for 10 minutes to absorb the culture to the medium. Agar wells (3 mm) in diameter were punched in the plates using a sterile gel puncture. Different concentrations of earthworm coelomic fluid were pipetted into the well and plates were incubated for 24 hrs in an incubator [19]. Zone of inhibition around the wells were recorded in mm.

## Results and discussion

### *Isolation of coelomic fluid from earthworm*

Nandhitha Madhusudhan *et al* [20] reported that the best method for isolation of coelomic fluid shows greater recovery by cold shock when compared to other methods such as heat shock method and electric shock method. When the earthworms were subjected to cold shock method, the essential proteins as well as enzymes were not prone to denaturation as in heat shock and electric shock, which may be the reason for the higher cell density observed.

Fig. 1. cold shock setup

The earthworm was collected and it was surface sterilized by using distilled water. Then it was air dried for 10 min. A bag of ice that fits over the funnel was placed above the worms. The decrease in temperature will induce the earthworm to release coelomic fluid in 15 -30 minutes. The coelomic fluid gets collected in 50 ml volumetric flask. The coelomic fluids were centrifuged immediately at 1000 rpm for 15

 

minutes. The cell free supernatant was collected and the protein concentration was estimated by Bradford's method.

### *Characterization of coelomic fluid proteins by SDS-PAGE method*

The coelomic fluid was collected and determined the molecular weight of different types of protein by SDS - PAGE. Investigators reported that the biological activities of the coelomic fluid of the earthworm *Eisenia foetida* could be related to some molecules with 33, 40, 42, 45 and 60 kDa molecular masses [21]. As one of the most vital defence components, antimicrobial peptides are now considered as one of the universal host defence tools of living organisms against microbial infection. Up to now, the molecular weight of antibacterial peptides are commonly found to be below 60 KDa [22]

In the present work, there had been five different protein bands that appeared. Among these, two proteins of 58 and 47 KDa were seen (Fig. 2). This is in consistent with the earlier findings. In their study, two proteins, of 40 and 45 KDa, designated as fetidins exhibited hemolytic and antibacterial activity Milochau *et al.* (1997) [23].

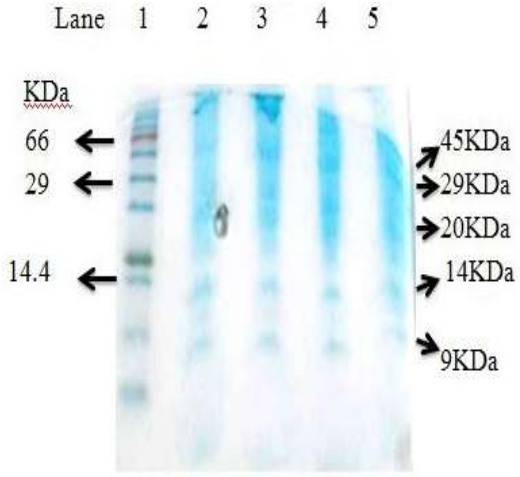


Fig. 2. SDS-PAGE analysis of proteins from earth worms coelomic fluid (Lane 1: Protein Marker, Lane 2: Crude coelomic fluid, Lane 3: Acetone precipitated crude coelomic fluid, Lane 4: Dialysate of crude coelomic fluid, Lane 5: Dialysate of acetone precipitated crude coelomic fluid)

### *Testing of anti-bacterial activity of coelomic fluid from earthworm*

Anti- bacterial activity of coelomic fluids of earthworms was done by dilution and agar diffusion test against three different bacterial strains. To test the anti- microbial activity of coelomic fluid, the inhibition zone formation around the coelomic fluid was observed and measured the diameter of that zone of inhibition range (Table 1).

Table 1. Antibacterial sensitivity of coelomic fluid

| Type of microorganism/ | Zone of Inhibition (mm) for Various Concentration Coelomic Fluid (mg) | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 |
| *Bacillus subtilis* | ±8 | ±10 | ±15 | ±18 | ±21 |
| *Staphylococcus aureus* | ±14 | ±17 | ±21 | ±24 | ±27 |
| *E. coli* | ±14 | ±18 | ±23 | ±25 | ±28 |

The zone of inhibition was recorded for various concentrations such as 10, 20, 30, 40 and 50 mg. There zone of inhibition slightly increased based on concentrations coelomic fluid against various bacterial strains. As a conclusion, the antibacterial activity shows that the coelomic fluids are effective in high concentrations for all bacterial strains.

The coelomic fluid of *Eisenia foetida* is demonstrated to possess an antimicrobial activity against *Aeromonas hydrophila* and *Bacillus megaterium* which are known as earthworm pathogens [24]. Numerous nanoparticles were synthesized using coelomic fluid proteins and antimicrobial activities were ananlysed recently [25] [26] [27] [28] [29]. Afterwards, Milochau *et al* (1997) [23] obtained two proteins, named Fetidins, from dialyzed coelomic fluid of earthworms and confirmed that the antibacterial activity was due to fetidins.

## Conclusions

Earth worms are able to protect themselves against invading microorganisms through their immune system. Earth worm plays a major role in the proper functioning of the soil ecosystem. It acts as scavenger and helps in recycling of dead and decayed plant material by feeding on them. Earth worm increases the soil remedies. The coelomic fluid prepared from earth worm *Eudrilus eugeniae* was tested by SDS-PAGE for antibacterial activities. The *E. coli* bacterium cultures were used for antibacterial testing maintained on nutrient agar slant. The minimum

 

inhibitory concentration was determined using agar diffusion method. The antibacterial activity of non-diluted coelomic fluid was most effective on bacteria compared to all diluted coelomic fluid. The minimum inhibitory concentration results indicate that earthworm coelomic fluid at a dose of 100 µl inhibited the bacterial growth. Hence earthworm coelomic fluid has a good potential to develop a new drug

## Conflicts of Interest

The authors declare no conflict of interest.

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