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Earthworm's coelomic fluid: extraction and importance

Soumya R Patil, * Pulikeshi M Biradar

Department of P. G. Studies in Zoology, Karnatak University, Dharwad, Karnataka, India

Abstract
The coelomic cavity of earthworms is filled with fluid derived from mesenchymal lining. The coelomic fluid consists of watery matrix, the plasma and a large number of coelomocytes having wide variety of functions, which plays a very important role in building innate immunity. The coelomic fluid is generally secreted by the earthworms for maintaining moisture in the body to help their physiological activities. It is generally expelled through the dorsal pores at the time of stress in response to mechanical and chemical irritation. There are various methods used for the extraction of coelomic fluid from earthworms, namely, warm water, electric shock, cold shock and heat shock methods. Out of these, cold shock method is most advantages over the other methods especially for earthworms. The coelomic fluid has many biological properties such as cytotoxic, proteolytic and anti-microbial, haemolytic, haemo-agglutinating, mitogenic activities etc. Several bioactive compounds have also been found in this coelomic fluid, exhibit a variety of biological functions.

Keywords: earthworms, coelomic fluid, coelomocytes, extraction methods

Introduction
Earthworms are metamerically segmented oligochaeta belongs to the phylum Annelida. They are negatively phototactic, soft bodied, true coelomates, saprophytic creatures of agro-ecosystem and major macro fauna of soil biota. Earthworms have also been living with the aid of their defensive mechanism since long time during the course of evolution and always face the invasion of microorganisms in their environment (Englmann *et al.,* 2004). Such studies have been continued for more than 50 years and then confirmed that earthworms have developed both humoral and cellular immunity mechanisms to fight against pathogenic microorganisms (Beschin *et al.,* 1998; Bilej *et al.,* 2001; Field *et al.,* 2004) [6, 5, 11].
Many invertebrates exhibit various immune mechanisms against different pathogens. Likewise, earthworm also prevails coelomocytes located in the coelomic fluid is responsible for both innate cellular immune functions such as phagocytosis and encapsulation against pathogenic microorganisms (Cooper *et al* 2001) [7] and humoral components includes lectin, pre-forming proteins, phenoloxidases and proteases nullifies antigenic material by agglutination and cytotoxicity etc. (Lange *et al*, 1999) [17].
The coelomic cavity is filled with fluid containing free wandering coelomocytes derived from mesenchymal lining. The transport of coelomic fluid between neibouring segments is ensured by channels comprised of sphincters within the septa. Each segment of the coelomic cavity is opened to the outer environment by paired nephridia and by one dorsal pore through which soluble metabolites and corpuscular materials respectively can be expelled out (Bilej *et al.,* 2000; Weidong *et al.,* 2003) [4, 23].
Many studies have been investigated about the composition of the coelomic fluid at an enzymatic level and were demonstrated that the presence of haemolytic, proteolytic and cytotoxic enzyme that are active against foreign cells and peptides (Bilej *et al.,* 1995; Yamaji *et.al.,* 1998; lange *et al*, 1999; Cooper and Roch, 2003) [3, 17, 16]. The bioactive compounds present in the coelomic fluid of earthworms have caused much attention in many scientists (Cooper, 2002; Yu Shen, 2010) [8]. Researchers have already investigated some features and activities of few enzymes that were isolated and purified from coelomic fluid.
Several other bioactive proteins have also been found in the coelomic fluid of earthworms, exhibit a variety of biological functions such as antibacterial (Valembois *et al.,* 1982) [21], haemolytic (Roch *et.al.,* 1981) [19], cytotoxic (Kauschke and Mohrig, 1987) [15] hemagglutinating (Roch *et.al.,* 1986) [20] activities. The biological and chemical nature of these compounds is responsible for such activities and has been studied extensively for more than two decades (Bileji, 1994; Valembois *et.al.,* 1986) [2]. This kind of investigations have been studied much in the earthworms *Eisenia fetida*, *Lumbricus terrestris* and *Dendrobaena venata* (Furlong *et al.,* 2002; Kalac *et al.,* 2002; Koening *et al.,* 2003) [12]. However, there is little knowledge about the metabolic complement of the coelomic fluid of earthworms.
Literature revealed that many aspects with respect to ecology, physiology, behaviour of earthworms have been studied much but very little has been known regarding composition of coelomic fluid, its importance and usage with respect to medicinal value (Reynolds and Reynolds, 1979) [18].

Earthworm coelomic fluid
The coelomic fluid is generally secreted by the earthworms for maintaining moisture to help their physiological activities such as respiration and burrowing activities. It consists of watery matrix, the plasma and a large number of coelomocytes. These coelomocytes play a very important role in building innate immunity of earthworms, are differentiated into four different types of immune cells such as amoebocytes, mucocytes, circular cells and chloragogen cells, which have different shape, size and have wide variety of functions.

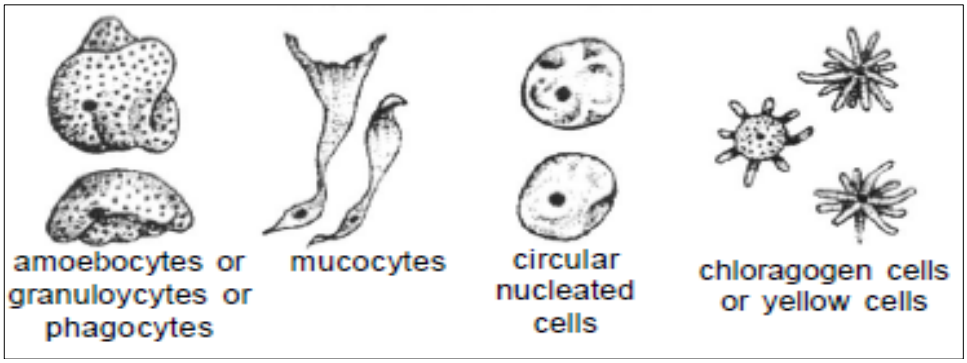

Fig 1

Amoebocytes: These cells number are usually more, which are large in size and spherical in shape helps mostly in removing harmful bacteria from earthworms. They are also known as granulocytes or phagocytes.

Mucocytes: These cells are elongated and its narrow end bears nucleus, they secretes mucous so as to keep the skin always moist for respiratory and other physiological functions.

Circular cells: These cells are nucleated, circular in shape and the functions of these cells are not known.

Chloragogen cells: These cells are found in large number; functionally have been described as trophocytes, which are taking part in upliftment of nutrients such as proteins, lipids and glycogens to different cells and organs of the body through coelomic fluid via circulation.

All these coelomocytes present in the coelomic fluid have their own functions such as pressuring, desiccation, promoting cutaneous respiration or producing protective measures against predators, regeneration, in circulating nutrients and mainly in defensive mechanism both in cell mediated and humoral immunity for the protection of earthworms.

Extraction of coelomic fluid

There are number of methods for collection of coelomic fluid by applying electric stimulation or by making rise or drop in temperature (Hideshi *et al.,* 2004: Weidong *et al.,* 2003) [23]. Likewise, through different methods, the body fluid is being collected along with enormous quantity of water which is known as vermiwash (Zambare *et al.,* 2008; Abdullah, 2008) [26, 1].

Many techniques have been developed so as to obtain coelomic fluid from earthworms for various biological activities etc. It is expelled through the dorsal pores at the time of stress in response to mechanical and chemical irritation.

The different methods of extraction of coelomic fluid by earthworms and their merits and demerits were highlighted here.

There are usually four methods are practicing now a days for the collection of coelomic fluid from earthworms namely

1. Warm water method
2. Electric shock method
3. Cold shock method
4. Heat shock method

1. Warm water method

Here, some quantity of worms (±15 grms) were taken, kept immersed under 25ml of warm water (45°C) and the whole fluid can be used as coelomic fluid (vermiwash) for further analysis or activities.

2. Electric shock method

Here also, same quantities of earthworms (±15 grms) were subjected to mild electric shock of about 5 volts for 30 minutes. The released fluid is considered as coelomic fluid.

3. Cold shock method

Same quantities of earthworms (±15gms) were subjected to cold shock by using ice cubes in a petriplates and the fluid is collected in a clean dry test tube. Through this method, we can able to produce 1.5 ml of coelomic fluid in 30 minutes.

4. Heat shock method

Similar to cold shock method, here ice cubes were replaced with hot water bag (55-60°C) with a different set of worms (±15 grms). Through this method, we can yield only about 0.5-0.25ml of coelomic fluid.

All the above methods of coelomic fluid extraction were subsequently employed for different experimentation depending upon the need. There was marked influence on the earthworms after their usage in different collection methods such as worms underwent either electric shock or heat shock method were almost dead or suppressed, this clearly indicates that the conditions for collection of fluid were of extremely stressful for the worms.

First two methods such as warm water and electric shock methods were not used much due to enormous quantity of water is used in the fluid and due to mortality problems respectively. In case of warm water method, worms were alive and active but, there was no increase in the volume of the fluid secretion, whereas the worms that were subjected to cold-shock method were alive and active, though, they also secretes larger volume (1.5ml) of fluid compared to other methods.

Merits and demerits of cold shock method

Cold shock method found to be the safest method to collect coelomic fluid from earthworms because of placing worms under the ice did not seems to be harmful as seen by their activeness and survivability of the worms after every time of fluid collections. The growth of the worms was found to be normal even after three rounds of fluid collection within a time period of one month. Even, there was no significant reduction in the volume of fluid in subsequent collections.

The fluid collected through this method is clearly brown in colour without any marked debris that was seen in the electric and heat shock methods. Here, used worms were in good conditions, even after the collection of coelomic fluid and can be easily cultured separately in cattle manure for subsequent collections after a time period of two weeks. Hence, this cold

shock method of fluid collection from earthworms is a novel method by using low temperature.

There are several advantages of cold shock method

1. In this method, the collection of coelomic fluid is easy & simple, involving no skilled labour and we get much concentrated coelomic fluid (vermin wash) here.
2. It does not require any apparatus such as the step down transformer/power eliminator etc.
3. Most importantly, worms were un-harmed and show normal activities even after several times of fluid collection.
4. Here, the coelomic fluid can be stored, packaged for transportation owing to its application or diluted at the user end.

Conclusions

The coelomic fluid consists of watery fluid released from dorsal pores of earthworms consists of large number of coelomocytes such as amoebocytes, mucocytes, circular cells and chloragogen cells, plays a very important role in both cellular and humoral immunity of earthworms in fighting against pathogenic microorganisms. The coelomic fluid contains several bioactive compounds such as proteins, exhibits a variety of biological functions such as antibacterial, anti-cancer, haemolytic, cytotoxic, hemagglutinating and proteolytic activities etc. As per comparisons of different methods used in the extraction of coelomic fluid from earthworms, cold shock method is a novel and beneficial method as it won't require any apparatus and more importantly used earthworms are unharmed and show normal activities even after collection of coelomic fluid several times.

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