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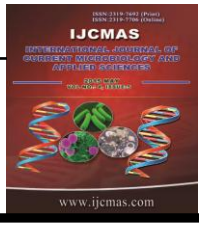

Original Research Article

A Simple Protocol to Isolate and Culture Coelomic Cells of Earthworms

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A B S T R A C T

The interest to study coelomocytes of earthworms as immunocompetent cell is increasing worldwide. The number and density of various coelomic cells may be species specific, and seasonal environmental stress may not be ignored. There may be variation in number and size of coelomocytes in relation to availability of nutritive compounds as well as their annual cycles. With this aim present study focused to develop a simple protocol to isolate and culture coelomic cells in various species of earthworms for immunocytological and other modern area of sciences.

Keywords

Coelomic cells; coelomocytes; amoebocytes; granulocytes; immune-competent cells of earthworms.

Introduction

Coelomic fluid of earthworms plays an important role in several physiological processes in earthworms including transportation of nutrients, metabolic intermediates and end products, respiratory gases and signaling molecules. The fluid comprises characteristic coelomic cells that regulates immune system and maintains hydrostatic skeleton in earthworms. There are three types of cells namely; amoebocytes, granulocytes and eleocytes have been observed by various workers (Cooper and Stein,1981; Hamed *et al*., 2002 and Adamowicz, 2005). The amoebocyte moves by pseudopodia, phagocytose foreign material; granulocytes contains various granules and vacuoles while eleocytes (chloragocytes) are characterized with presences of glycogen particles, lipid droplets and granules. These coelomic cells (coelomocytes) maintain innate cellular immune responses (phagocytosis, inflammatory processes, graft rejection and coagulation of coelomic fluid) and humoral immune responses (secretion of lysosome, agglutinine, phenoloxidases, peroxidases and antimicrobial factors *viz*., fetidin, lysenin, eiseniapore, coelomic cytolytic factors). Thus coelomic cells are considered as best model to study immunity mechanism. Since limited protocols (Madhusudan *et al*.,2009) have been developed to isolate and culture coelomic cells. Present study is aimed to standardized simple method for *in vitro* culture of coelomic cells.

Materials and Methods

Study Undertaken with following Protocol

Reagents: Extrusion buffer; Ethyl alcohol; LBSS solution; DMEM; FBS.

Composition of Extrusion Buffer

a) NaCl 71.2mM
b) Ethanol 5%
c) Guaicol-glycerol-ether 50.4mM
d) EGTA (Ethylene glycol tetraacetic acid**)** 5mM (EDTA, Ethylene diamine tetra acetic acid may used if EGTA is not available*)* and maintain pH 7.3. (or taken 0.093 g EGTA or EDTA in 50 ml distilled water and dissolved with maintaining pH 7.3; special care required to maintain pH).

A Method of Preparation of Guaicol-glycerol-ether

Add 92.09 g Guaicol and 124.14 g *Glycerol in 74.12 ml Ether.

If ether was not available 1g Guaicol and 1 g *Glycerol in 50 ml distilled water were added (this ratio also worked).

* Preferred to weigh glycerol.

Method of Preparation of Extrusion Buffer

Added 0.208g NaCl in 5% ethanol (2.5 ml ethanol in 50 ml distilled water) and add 0.5 ml of Guaicol-glycerol-ether mix. Finally added 1ml of EGTA or EDTA buffer and made up volume 50ml with distilled water.

Composition of LBSS

a) NaCl 71.5mM
b) KCl 4.8mM
c) $MgSo_4.7H_2O$ 1.1mM
d) KH_2PO_4 0.4mM, pH 7.3

Method of Preparation of LBSS (Lumbricus Balanced Salt Solution)

0.417 g NaCl + 0.035 g KCl + 0.018 g $MgSo_4.7H_2O$ +0.005 g KH_2PO_4 + 0.004 g NaH_2PO4 + 0.035 g NaHCO3 were added in 100 ml distilled water and maintained pH 7.3.

Method of Preparation FBS spiked DMEM

10 ml Fetal Bovine Serum(FBS) were addded in 90 ml Dulbecco's Modified Eagle Medium(DMEM).

Procedure

Thoroughly washed worm in running tap water before rinsing in distilled water and placed on wet cotton to ensure complete defecation in order to avoid contamination during harvesting of coelomocytes. After 2-3 hrs, wipe the worms with cotton wool soaked with 70 % ethyl alcohol to avoid any further contamination.

Placed surface cleaned worms in sterile petridish containing cold extrusion buffer for one-two minutes only.

Coelomic fluid was excreted out through dorsal pores due to external stress condition. After collection of coelomic fluid in cold extrusion buffer, worms were released in soil. Pipette out excreted coelomic fluid into tubes filled with LBSS solution and centrifuge at 4°C for 5 min.

Loose pellets of coelomocytes were formed, washed 2-3 times with cold LBSS solution. Washing was done with use of DMEM solution when LBSS was not available. Cell counted with trypan blue dye exclusion on heamocytometer. It was maintained close to 10^7/ml.

Isolated coelomocytes were load in petridish or cell culture flask with DMEM supplemented with 10% FBS. Incubated for 3 days in CO_2 incubator.

Viability of cells was recorded using haemocytometer and examined three types of coelomic cells (amoebocyte, granular amoebocyte and eleocyte) in phase contrast/fluorescence microscope (Fig 1). Sometimes, antibiotics like Penicillin or Streptomycin at 10-20 µg/ml was also used to avoid contamination.

Results and Discussion

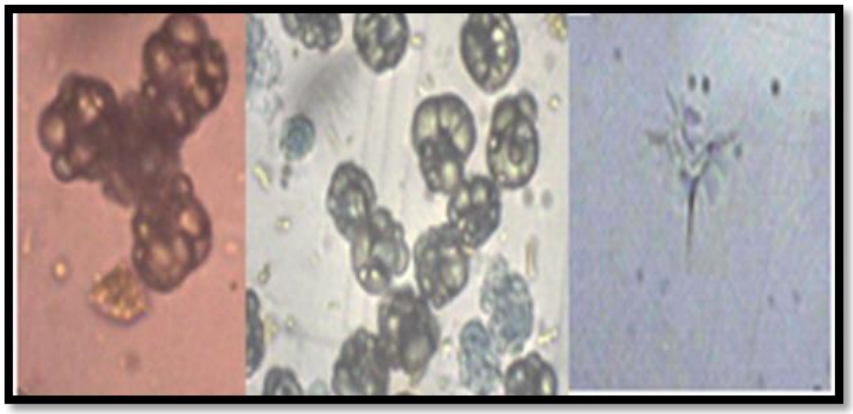
The coelomocytes isolated and cultured with present protocol may be useful for analysis of immune-cytochemical and other genotoxicity studies. The developed protocol was used to isolate and culture coelomic cells of different species of earthworms (*Eisenia foetida*; *Eudichogaster prashadi*; *Perionyx sansibaricus*; *Eudrilus eugeniae* : Table 1) and were reported worked well in all species of earthworms.

Table.1 Coelomocyte count in different species of earthworms at the time of extraction and after three days incubation

Earthworms		**Coelomocytes**							
Species	**Body weight (g)**	**ECE ($X10^6$)/g**	**GAE ($X10^6$)/g**	**HAE ($X10^6$)/g**	**TCE ($X10^6$)/g**	**ECI ($X10^6$)/g**	**GAI ($X10^6$)/g**	**HAI ($X10^6$)/g**	**TCI ($X10^6$)/g**
Eisenia foetida	0.60 ± 0.04	2.7±0.5	1.7±0.4	0.6±0.1	5.0±0.8	3.9±0.4	4.0±0.3	0.3±0.01	8.2±0.6
Eudichogaster prashadi	1.18±0.16	1.0±0.2	2.8±0.8	0.2±0.1	4.0±0.6	4.8±0.1	1.2±0.4	0.2±0.02	6.2±0.6
Perionyx sansibaricus	0.40±0.02	0.8±0.1	1.9±0.2	0.2±0.1	2.8±0.7	2,6±0.1	1.9±0.1	0.1±0.01	4.6±0.4
Eudrilus eugeniae	2.10±0.16	2.0±0.2	1.0±0.7	0.4±0.2	3.4±0.3	2.8±0.2	1.6±0.2	0.1±0.01	4.5±0.3

ECE, eleocyte count at the time of extraction; GAE, granular amoebocyte at the time of extraction; HAE, hyaline amoebocyte at the time of extraction; TCE, total cell count at the time of extraction; ECI, eleocyte count at the time of incubation; GAI, granular amoebocyte at the time of incubation; HAI, hyaline amoebocyte at the time of incubation; TCI, total cell count at the time of incubation.

Fig.1 A view of different coelomic cells of *Eisenia foetida* viz., eleocytes; granulocytes; amoebocytes



A uniform classification of coelomocyte of different species of earthworm is little difficult (Adomowicz and Wojtaszek, 2001) as they exists in various functional states and stages of maturation. Broadly on the basis of morphology, Cooper and Stein (1981) described two type of coelomocytes namely, amoebocytes (hyaline and granular) and eleocytes. The origin and relationship of coelomocytes are not yet completely known. The number and composition of the coelomocyte depends on exogenous (environmental) as well as endogenous (biotic, life cycle) factors. The present study could be used to rapid isolation and culture of coelomocytes of earthworms for further studies.

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