IJASRM
International Journal of Advanced Scientific Research and Management, Volume 4 Issue 7, July 2019
www.ijasrm.com
ISSN 2455-6378

The regenerative efficacy of native earthworm, *Drawida pellucida pallida* and exotic earthworm, *Eudrilus eugeniae*

Akila Moorthy K[1], Rini Joseph[1], Deepthi M P[1], Kathireswari P[1*] and Saminathan K[2*]

[1]Department of Zoology, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu- 641029, India

[2*]Department of Chemistry, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu- 641029, India

Abstract

Many organisms have the potential to regenerate its lost tissues to some extent from the healing of wounds to replace the whole organs. Epigeic or surface dwelling earthworms are mostly subjected to predator attacks or by soil cultivators during ploughing. Hence, the nature has blessed them with the power of regeneration. The regenerated tissue, forms wound healing to replace the entire organs. Our present study was aimed to observe the regenerative efficacy of native and exotic epigeic earthworms *Drawida pellucida pallida* and *Eudrilus eugeniae* respectively. The amputation, were done on earthworms at anterior and posterior regions, organogenesis at the time of regeneration were studied in *Eudrilus eugeniae* and *Drawida pellucida pallida.* It was observed that the earthworm regenerates its functional parts mouth and anus within five to six days. The regenerative potential rate was observed higher for exotic earthworm *Eudrilus eugeniae* when compared to native earthworm *Drawida pellucida pallida*. The exotic species *Eudrilus eugeniae* have high growth, survival capacity and high regenerative potential than the native species *Drawida pellucida pallida*.

Keywords: *Regeneration, earthworm, Eudrilus eugeniae, Drawida pellucida pallida, organogenesis, amputation.*

1. Introduction

Regeneration is a remarkable phenomenon entailing re-growth of a detached or lost body part. Earthworms are morphologically more complex than hydra and planarians, which shows variable degree of regeneration [1]. Tissue regeneration, or the restoration of lost body parts defined by [2], and it is commonly seen in members of the Annelida. Regeneration behavior was extensively observed within the phylum with some enchytraeid oligochaetes can able to regenerate into complete organisms from fragments comprised of a few segments [3], other taxa amongst the oligochetes and polychetes possessing different degrees of ability to regenerate anterior and posterior segments, with posterior regeneration particularly prevalently observed and Hirudinea apparently incapable of any segment replacement [4].

The regenerative capacity can also be influenced by environmental factors such as temperature and nutrition. [5] reported that the earthworms cultured at 25 ^{0}C regenerated faster than those kept at 30 ^{0}C and 20 ^{0}C and the sexual activity can also influence rates of regeneration.

There are several reasons that the earthworm provides a unique valuable model to investigate the mechanism of regeneration. Firstly, the earthworms can able to regenerate rapidly. Secondly, the complete regeneration of head and tail requires reformation of various tissues and organs, including a central nervous system, heart, clitellum, blood-vascular system, testis, ovary, intestine, nephridia and setae. Thirdly, earthworm regeneration is bidirectional [6]

The present study was undertaken to elucidate the phenomenon of the regeneration of the mouth and anus in the earthworm, exotic *Eudrilus eugeniae* and native *Drawida pllucida pallida.* Uniquely, the earthworm regenerated its functional mouth and anus by the 6th day and during the process of organogenesis, the earthworm resorted to starvation behavior for the first 5 days. This adaptive behaviour of starvation triggered the gut microflora to produce riboflavin, which is the key factor in inducing regeneration[7].

2. Materials and Methods

(1) Collection and identification of earthworms

The earthworm *Eudrilus eugeniae* and *Drawida pellucida pallida* were collected from vermicompost unit of Department of Zoology, Kongunadu Arts and Science College and cultured according to the protocol of [8]. The sexually matured fully clitellated earthworms were selected from the vermibed. The earthworms were cultured in cowdung medium and with leaf litters as substrate.

Drawida pellucida pallida were obtained from kitchen waste deposition in Forest College and Research Institute (Longitude of 11.19' N and latitude of 77.56' E) Mettupalayam, Tamil Nadu. The samples were collected with the soil and they were maintained in a vermibed prepared in college.

(2) Wound healing and regeneration analysis

In order to observe the wound healing and regeneration processes, in exotic and native earthworms *Eudrilus eugeniae* and *Drawida pellucida pallida* were amputated at pre-clitellum and post-clitellum part and observed the development of head and tail regions. Before amputation the, earthworms were rinsed with de-ionized water. Amputations were done using a sterile blade. The amputated earthworms were kept in a clean sterile beaker provided with wet filter paper to maintain moisture content for the survival of earthworms. The amputated regions of earthworm segments were analyzed regularly using Stereo Zoom microscope attached with camera and observed regions were photographed.

(3) Analyzing the functional part Mouth and Anus after amputation

The amputated earthworms were kept in a beaker with wet tissue paper according to the protocol [7]. The feeding behavior were observed by the colour changes along the sides of the tissue paper, if earthworm starts feeding the colour of the filter paper changes from white to pale brown and the tissue paper moving through their gut can be easily analyzed using the stereo zoom microscope (Magnus) and excretion analysis done by the presence of brown fecal pellet formation.

3. Results and Discussion

Annelids exhibit a wide-range of variations in regenerative ability [9] which has much higher phylogenetic and metameric structure than the lower organism's hydra and planarians. Annelid's regeneration was mainly achieved by dedifferentiation and cell activation as vertebrate's regeneration, therefore annelids would be more suitable than hydras or planarians as an experimental material for exploring base to enhance the regeneration ability of vertebrates [12].

Earthworms are segmented annelids with anterior segmental region starts with a ventrally situated mouth called prostomium, followed by a long digestive canal called intestinal region connected to several organs and ends up with a dorsally located anus. The earthworm regeneration mainly occurs by de-differentiation and re- differentiation of cells, without any contribution of totipotent stem cells [11]. The regenerative potential rate were analyzed and compared between two different species of epigeic earthworms, an exotic earthworm *Eudrilus eugeniae* [E] and native earthworm *Drawida pellucida pallida* [D] belongs to the family Eudrilidae and Moniligastridae. Both the samples were collected and maintained in the vermibed with cowdung. In order to compare the regeneration potential of earthworm, healthy adult earthworms were selected from both the species maintained from vermibed. For experimentation the body segments of these earthworms were classified into two regions based on the reproductive region clitellum, namely (1) Pre-clitellar (before clitellum) and (2) Post-clitellar segment (after clitellum). The pre-clitellum segments in both *Eudrilus eugeniae* and *Drawida pellucida pallida* consist of all vital organs such as prostomium, mouth, brain and the post-clitellum region has intestine and anus. In first group two earthworm species were amputed at pre-clitellar region of 3rd and 5th segments of *Eudrilus eugeniae* [E1] and *Drawida pellucida pallida* [D1] and they were studied by Pre clitellar head development and pre clitellar tail development. In second group were classified into post- clitellar region of 30th and 68th sements of *Eudrilus eugeniae* [E2] and *Drawida pellucida pallida* [D2] and they were studied by Post clitellar head development and post clitellar tail development. The worms were surface sterilized using distilled water and amputated worms were maintained in a rearing bed with sterile wet tissue paper and the regeneration ability was regularly examined using stereo zoom microscope, photographed and documented.

Soon, after amputation coagulation was noticed within 1 to 2 minutes at the wounded sites of all amputated earthworms. Then the wound closures occurred in anterior and posterior within 24 h in both the species. After amputation the complex process of regeneration starts with the wound healing process, which incorporates the formation of blastema explained by [10], then successful development of organogenesis of lost organs [7]. The wound healing happens by the superficial epiblasts originating from undifferentiated epidermal cells [12] and skin epithelium has a main role in wound healing [13]. The blastema formation during regeneration was

IJASRM International Journal of Advanced Scientific Research and Management, Volume 4 Issue 7, July 2019
www.ijasrm.com
ISSN 2455-6378

achieved by the proliferation of longitudinal cell layer [14].

On the 3rd day the regenerated blastema was found in both the anterior and posterior regions of head and tail development were observed under stereo zoom microscope. The developing blastemas appeared as a colorless or less pigmented mass of freshly formed tissues. The normal segments will be in dark colour with high pigmentation and the new regenerative tissues can be easily analyzed by the lighter colour during early weeks of regeneration. And many angiogenic blood vessels were observed in the newly developed blastema in *Eudrilus eugeniae* and *Drawida pellucida pallida* on 3rd day. The structural development were observed in day 3 blastemas of the anterior and posterior regions of regenerating earthworms were subjected to histological analysis by [7] states that the blastema cells were newly formed and the epithelial cell layer (ECL) and circular muscle layer (CML) were not found in the developed regeneration blastema.

The development of mouth and anus were observed on the sixth day using stereo zoom microscopy. But the survival rate of *Drawida pellucida pallida* was very less when compared with the exotic species *Eudrilus eugeniae*. It was observed that the development of mouth opened ventrally and anus opened dorsally. The segment formation also observed in both the species, when compared with the third day the vascular networks were more visible in newly formed regions, since the pigmentation was less in regenerated region. At the seventh day it was observed that the rapid growth and pigmentation in the newly generated head and tail, and the regenerated part resembled the mouth and anus.

The subjects of regeneration upon amputation at pre-clitellum segments of Eudrilus *eugeniae* were studied in detail [15]. Morphological studies have revealed that the presence of the nervous system is required for regeneration to proceed [16, 17]. New theories about the role and amount of regeneration of different parts of earthworm may ultimately have to be explained at the molecular level. For example, hox genes have been found to be expressed during the process of regeneration of parts of vertebrates and invertebrates [18]. To study the full recovery of mouth the earthworms were only provided and maintained in wet tissue paper. The body colour of earthworm changes when it starts consuming the white tissue paper and the nature of the gut material can be observed under stereo zoom microscope. The excretion process were observed by the excretion of fecal pellets confirms the normal functioning of anus.

The wound healing pattern was observed to be similar for both the earthworm species which amputated at the pre and post-clitellum segments. But the survival rate and regeneration capacity were observed more in exotic *Eudrilus eugeniae* when compared with native *Drawida pellucida pallida*. In *Eudrilus eugeniae* the mouth and anus development were observed in all the dissected samples of pre and post clitellar head and tail, but in *Drawida pellucida pallida* the development and the survival of earthworms were observed only in post clitellar head and tail development. [1] Explained that the posterior regeneration of earthworm is much more than anterior regeneration.

4. Tables and Figures

Figure. 1 Showing the earthworm species *Eudrilus eugeniae* and *Drawida pellucida pallida*

Figure. 2 Showing the amputation of earthworm and introduced beaker with wet tissue paper .

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ISSN 2455-6378

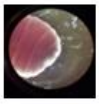
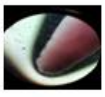
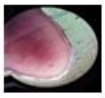
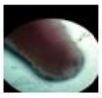
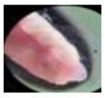
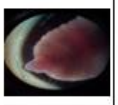
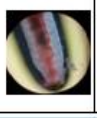
Days	Pre-Clitellum Head Development	Pre-Clitellum Tail Development	Post-Clitellum Head Development	Post - Clitellum Tail Development
Day 2				
Day 3				
Day 4				
Day 5				
Day 6				
Day 7				

Table. 1 Showing the images of regenerative stages in exotic earthworm *Eudrilus eugeniae* .

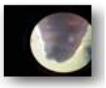
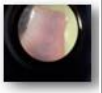
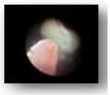
Days	Pre-clitellum Head development	Pre-clitellum Tail development	Post-clitellum Head Development	Post- clitellum Tail development
Day 2		No Regeneration		
Day 3		No Regeneration		
Day 4	Dead	Dead		
Day 5	Degraded	Degraded		
Day 6	Degraded	Degraded		
Day 7	Degraded	Degraded		

Table. 2 Showing the images of regenerative stages in native earthworm ***Drawida pellucida pallida***.

5. Conclusions

The experiments reported clearly that the earthworm, *Eudrilus eugeniae*, has high regenerative ability, certain well- defined survival and regrowth capacities and strategies after the amputation of various numbers of anterior or posterior segments than the native species *Drawida pellucida pallida*. In general, the exotic species *Eudrilus eugeniae* had the greatest and fastest survival rates when the segments were amputated. Further research is needed to clarify the actual mechanism of regeneration and it's variation. Although the phenomenon of regeneration has attracted the attention of earthworm biologist, adequate knowledge of its mechanism is lacking.

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