Biochemistry and Biophysics Reports 27 (2021) 101092

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

de novo transcriptome profile of two earthworms *Lampito mauritii* and *Drawida calebi* during regeneration

Ranjan Sahu [a], Sanjat Kumar Sahu [b], Sudhansu Sekhar Nishank [a,*]

[a] *Post Graduate Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, 751004, Odisha, India*
[b] *Dept. of Environment Science, Sambalpur University, Jyoti Vihar, Burla, 768019, Odisha, India*

ARTICLE INFO

Keywords:
RNA
Sequencing
Transcriptome
Regeneration
Earthworm
Gene

ABSTRACT

Earthworms have remarkable ability to regenerate its tail and head region. However the list of genes expressed in this regeneration process has been less explored baring a few species. The current study involves the *de novo* transcriptome sequencing of intact tail and regenerating tail (15 day post amputation) of earthworms belonging to two different genera *Lampito mauritii* (Kinberg, 1867) and *Drawida calebi* (Gates, 1945). This study contains one *de-novo* and one reference based transcriptome analysis each from one genus of two earthworm genera. From a total of 119.92 million (150 × 2) reads, 112.95 million high quality adapter free reads were utilized in analysis. Assembly of high-quality reads was performed separately for *Lampito mauritii* (LM sample) and *Drawida calebi* (DC sample) that resulted in 66368 and 1,61,289 transcripts respectively. About 25.21% of transcripts were functionally annotated for DC sample and 38.27% for LM samples against Annelida sequences. A total of 239 genes were expressed exclusively in regenerated tissue compared to intact sample in DC whereas about 241 genes were exclusively expressed in regenerated tissue of LM compared to its intact sample. Majority of genes in *Drawida* and *Lampito* were dedicated to immune response, maintenance of cytoskeleton, resisting oxidative stress and promoting neuronal regeneration for cell-cell communication during tail regeneration.

1. Introduction

Earthworms are one of the most important soil animals that maintain soil fertility and sustainability. In addition to their role in soil formation, they contribute to the composition and functioning of soil ecosystem with varying species diversity because of which they are known as ecosystem engineers. These earthworms have rapid rate of regeneration of their body parts if lost. They show both whole body and structural regeneration [1]. The mechanism of regeneration has been well studied in many members of annelids such as some fresh water oligochaetes and polychaetes, including earthworm where these organisms can replace segments amputated along the body rapidly and become virtually indistinguishable from normal adults within a short period. During regeneration, dedifferentiation and reprogramming of cells at amputed site takes place, where cells in wounds are degraded by phagocytes that migrate to site of amputation. The undamaged cells in wounds dedifferentiate and lose specialization and proliferate to form a conical bud leading to final differentiation for formation of various tissues [2].

The molecular mechanism of regeneration in animals has enabled biologist to understand basic mechanism of body pattern formation, maintenance of cell polarity, organ symmetry. Recent advances in sequencing techniques particularly transcriptome sequencing by next generation sequencer (NGS) and use of RNA interference techniques have revealed a vast information on these aspects in regenerating animals particularly planarians, hydra and earthworms [3,4]. The transcriptome profile of planarians during regeneration shows involvement of the *Wnt* signaling in tail regeneration and genes encoding predicted inhibitors of *Wnt* such as *FRP-1* and *notum* are involved in anterior body part regeneration. The dorsal ventral axis is regulated by *Bmp* signaling. The *bmp, admp* genes promote ventralization, *nog-1* gene promotes dorsalization [4,5].

In the earthworm *Eisenia fetida*, upregulation of three labial genes (Pex-lab01, Pex-lab02 and Pex-lab03), and distal-less gene induces head regeneration. During regeneration, dedifferentiation and reprogramming are found to be regulated by upregulation of five pluripotent factor genes (*Sox2, Oct4, Nanog, Lin28* and *Cmyc*) [2]. During entire period of regeneration, five super families of *Sox, Pax, Wnt, Klf, Hox* show higher expression of which *Pax* gene helps in bud/blastema formation and Wnt gene stimulates various signaling pathways in cell for formation of body axis. These six super families are thought to induce bud formation and

* Corresponding author.;
E-mail addresses: sahusanjat@rediffmail.com (S.K. Sahu), nishank25@gmail.com, nishankss.zool@utkaluniversity.ac.in (S.S. Nishank).

https://doi.org/10.1016/j.bbrep.2021.101092
Received 14 April 2021; Received in revised form 28 July 2021; Accepted 29 July 2021

remodeling of blood vessels, fats, nerves and muscles during regeneration in *Eisenia fetida* [2,6].

There have been many studies on the transcriptome profile of few earthworms such as *Eisenia fetida* and *Dendrobaena octaedra* [6,7]. Although, in Indian circumstances, transcription profile of *Eisenia fetida*, an exotic species has been reported during regeneration, there is lack of studies on transcriptome profile of many indigenous earthworms such as *Drawida calebi* (Gates, 1945) and *Lampito mauritii* (Kinberg, 1867) abundantly found in both cultivated and uncultivated land [8].

The present study is the first report (*de novo* transcriptome sequencing) to give information on transcriptome profiles of two indigenous earthworms *Drawida calebi* (Gates, 1945) and *Lampito mauritii* (Kinberg, 1867) during regeneration at stage of intact tissue and regenerated tissue at fifteen days post amputation (15dpa) so as to find out list of genes involved during regeneration in both genera of earthworms.

2. Methods

2.1. Sample preparation

Adult earthworms *Lampito mauritii* and *Drawida calebi* were collected from campus of Utkal University, Bhubaneswar after identification of these species using key features [8]. They were cultured separately in earthen pot containing cow dung, chopped dried leaves, vegetable scrapings and soil for one month. Five earthworms of equal lengths from each genus were taken out and placed in ice containing water kept in Petri dish and cut at seven segments posterior to clitellum. These amputed earthworms were kept in their respective soil pots for regeneration of tissue (Fig. 1 and Fig. 2). After fifteen days the regenerated tissue of all five earthworms from each genus at amputed site (designated as test sample) were collected, pooled together for RNA isolation and sequencing. Similarly in another group of five individuals from other genus, posterior portion of earthworms was amputed at seven segments posterior to clitellum and was collected (designated as control), pooled together for RNA sequencing. All pooled samples were collected in RNA Later solution (Qiagen) as per its prescribed protocol (Fig. 1 and 2).

2.2. cDNA library preparation and sequencing

RNA sequencing libraries were prepared with Illumina-compatible NEBNext® Ultra™ II Directional RNA Library Prep Kit (New England BioLabs, MA, USA) following its protocol. About 25.21% of transcripts were functionally annotated for *Drawida calebi* (DC) sample and 38.27% for *Lampito mauritii* (LM) samples against Annelida sequences. Reference based analysis was done for *Drawida calebi* regenerated (DC_RE) and *Lampito mauritii* regenerated (LM_RE) samples using Master Unigenes as reference. Sequencing for 150 bp length paired-end (PE) reads was performed in an Illumina HiSeq sequencer to produce on an average of 29.98 million raw sequencing reads at Genotypic Technology's Genomics facility, Bangalore (India) [9] (Fig. 3) (as supplementary file).

2.3. De-novo assembly and sequence clustering

Processed reads were assembled using graph based approach by rnaSPAdes [10] and clustering of assembled transcripts was performed by CD-HIT-EST [11]. Processed reads from all four libraries were aligned back to the final assembly using Bowtie2 [12] with end to end parameters.

2.4. Differential expression analysis and annotation

DESeq [13], a R package was used for differential expression calculation. Multiple databases (Uniprot, NCBI and KEGG pathway) were used for functional annotation of the transcripts. Clustered transcripts were annotated using homology approach to assign functional annotation using BLAST tool against "Annelida" data from the Uniprot database containing 80052 protein sequences. Transcripts were assigned with a homolog protein from other organisms, if the match was found at e-value less than e−5 and minimum similarity greater than 30%. Pathway analysis was done by using KAAS Server [14]. An annelid *Helobdella robusta* was considered as reference organisms for pathway identification. Simple Sequence Repeats (SSR) were identified in each transcript sequence using MISA [15] perl script. Simple repeat of motif length ranging from monomer to hexamer were identified with recommended default parameters of MISA.

2.5. Validation of RNA -Seq data by quantitative realtime PCR (qRT-PCR)

About 16 unigenes were selected for confirmation of RNA-Seq (quantification) data by qRT-PCR using SYBR Green chemistry (TB Green Premix Ex Taq II (Tli RNaseH Plus) (TAKARA #RR820A) in

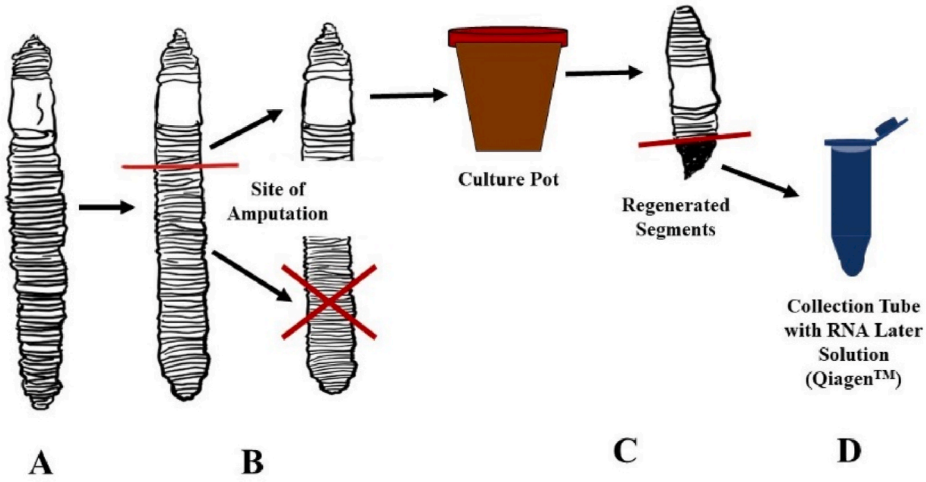

Fig. 1. Regeneration analysis scheme for Lampito mauritii and Drawida calebi. A. intact earthworm, B. site of amputation and selection of segments for culture, C. collection of regenerated part, D. preservation of regenerated tissue.

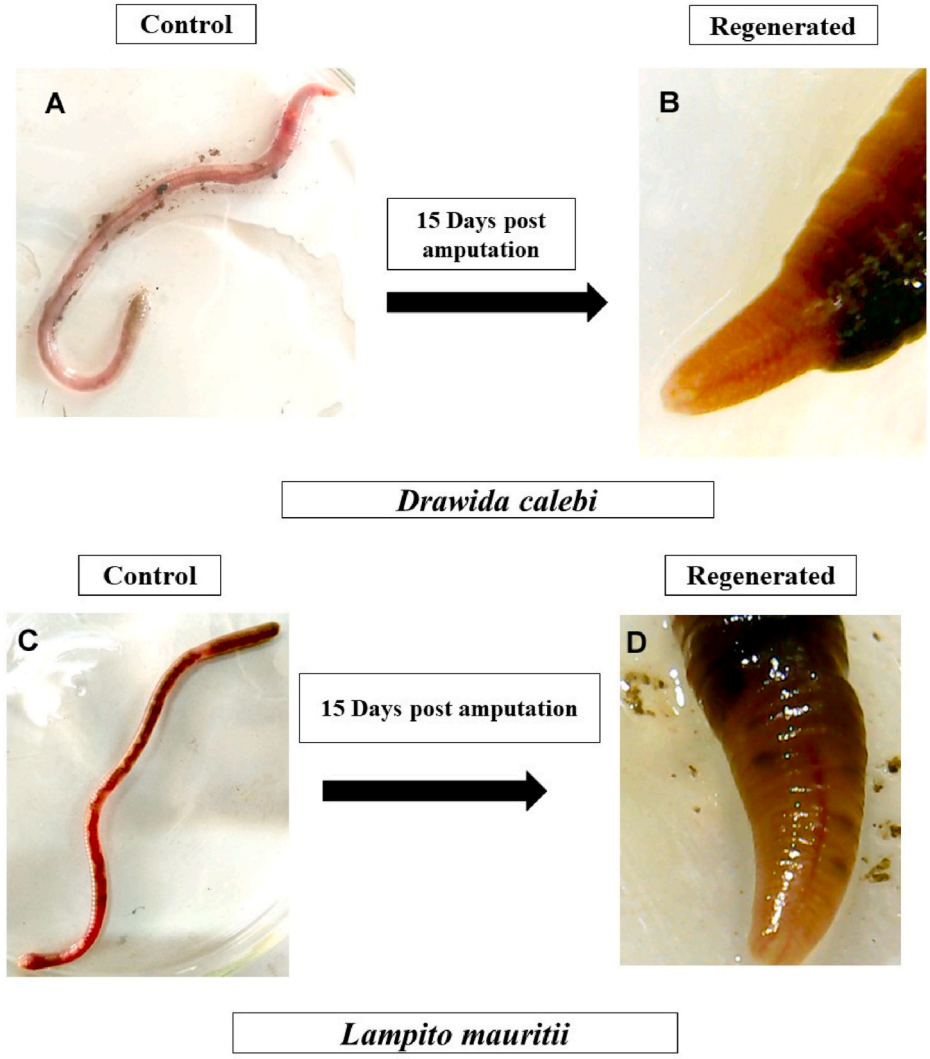

Fig. 2. Control (intact) and regenerated earthworm species, A. intact Drawida calebi, B. regenerated Drawida calebi, C. intact Lampito mauritii, B. regenerated Lampito mauritii.

Stratagene mx3005 P instrument (Agilent Technologies, USA). The dissociation curve analysis was performed after amplification for primer specificity. The mean Ct value of technical replicates was used to calculate the relative expression level of genes. The relative quantification of genes were analyzed using standard $2^{-\Delta\Delta Ct}$ as described by Pfaffl (2001). Comparison of gene expression between intact tissue and regenerated tissue was carried out using reference gene LM_28644 (protein afadin) in LM group and elongation factor 1 alpha in DC group. Primers specific to unigenes were designed by Primer3 plus software. The sequence of primers are described in Table 1 (supplementary file).

3. Results

3.1. Read statistics

The raw data pertaining to sequencing of intact and regenerated tissue of both DC and LM samples has been submitted to NCBI (Submission ID SUB5940071, BioProject ID PRJNA553867). An average of 28.23 million reads were used for the downstream analysis after preprocessing (Table 2) (supplementary file). For every sample an average of 93.90% of high quality data was retained.

3.2. Assembly statistics

The assembly of high quality reads was performed separately for LM and DC samples resulting in 66368 and 1,61,289 transcripts. Sample wise transcripts were merged and further clustered into 2,26,895 transcripts with an average length of 953 bp and N50 of 1430 bp. (Table 1).

3.3. Differential expression analysis

Heatmap was generated for top 20 up and down regulated transcripts. Following differential gene expressions (DGE) comparison were performed:DC_Vs._DC_RE and LM_Vs._LM_REDC_Vs._DC_RE and LM_Vs._LM_RE; In DGE reports, sample wise expression values were library normalized values between samples being compared. Normalized expression of a given transcript may change across different comparisons as the library size was not same for all DGE combination. Fold changes were calculated based on comparison of transcript profile between DC_RE and LM_RE. (Table 4, Table 5 and Table 6)(supplementary table). There were contrasting outcomes in the expression of genes between regenerated tissue and intact tissue in both *Drawida* and *Lampito* samples. (Fig. 4 and 5 as supplementary file).

Table 1
Assembly statistics of transcripts.

	DC	LM
Number of transcripts identified	1,61,289	66368
Maximum Contig Length	16400	28141
Minimum Contig Length	300	300
Average Contig Length	839.8	1225
Median Contig Length	323	907
Total Contigs Length	13,54,57,948	8,13,03,167
Total Number of Non-ATGC Characters	1,04,101	46741
Contigs≥500 bp	85783	40527
Contigs≥1 Kbp	37867	24008
Contigs≥10 Kbp	30	79
N50 value	1126	2143
	Master_Unigene[a]	
Number of transcripts identified	2,26,895	
Maximum Contig Length	28141	
Minimum Contig Length	300	
Average Contig Length	953.7 ± 1023.6	
Median Contig Length	2878	
Total Contigs Length	21,63,82,458	
Total Number of Non-ATGC Characters	1,50,842	
Contigs≥500 bp	1,26,091	
Contigs≥1 Kbp	61838	
Contigs≥10 Kbp	109	
N50 value	1430	

[a] Unigenes are de-duplicated transcripts by clustering.

3.4. Validation of differential gene expression through quantitative RT-PCR analyses

About five up-regulated genes and five down regulated genes of DC, DC_RE along with three up-regulated genes and three down-regulated genes of LM, LM_RE were randomly selected for validation by qRT-PCR. It was found that expression level of these up-regulated and down-regulated genes by qRT-PCR showed concordance with that of genes obtained from RNA sequence data generated by next generation sequencer (NGS) supporting the RNA-Seq results (Table 7) (Supplementary table). In the present study, the house keeping gene was Afadin in LM, LM_RE samples and EF-1-α (elongation factor 1 alfa) in DC, DC_RE samples.

3.5. Annotation

About 25.21% of transcripts were functionally annotated for DC sample and 38.27% for LM samples against Annelida sequences. Most abundant 10 terms from each category are represented as donut chart for DC and LM (Fig. 3, A-B).

Analysis of top hit species distribution data (similarity with other organisms based on sequence homology) for the transcriptome datasets of earthworm LM and DC samples identified the marine polychaete annelid worm, *Capitella teleta* (62.3% in LM and 62.9% in DC), *Helobdella robusta* (33% in LM and 32% in DC) a freshwater leech, *Platynereis dumerilii*, a polychaete ragworm (2.4% in LM and 2% in DC), followed by *Hirudo verbena*, a marine leech (0.76% in LM and 0.77% in DC) although similarity with *Eisenia fetida* is 0.34% in LM sample but

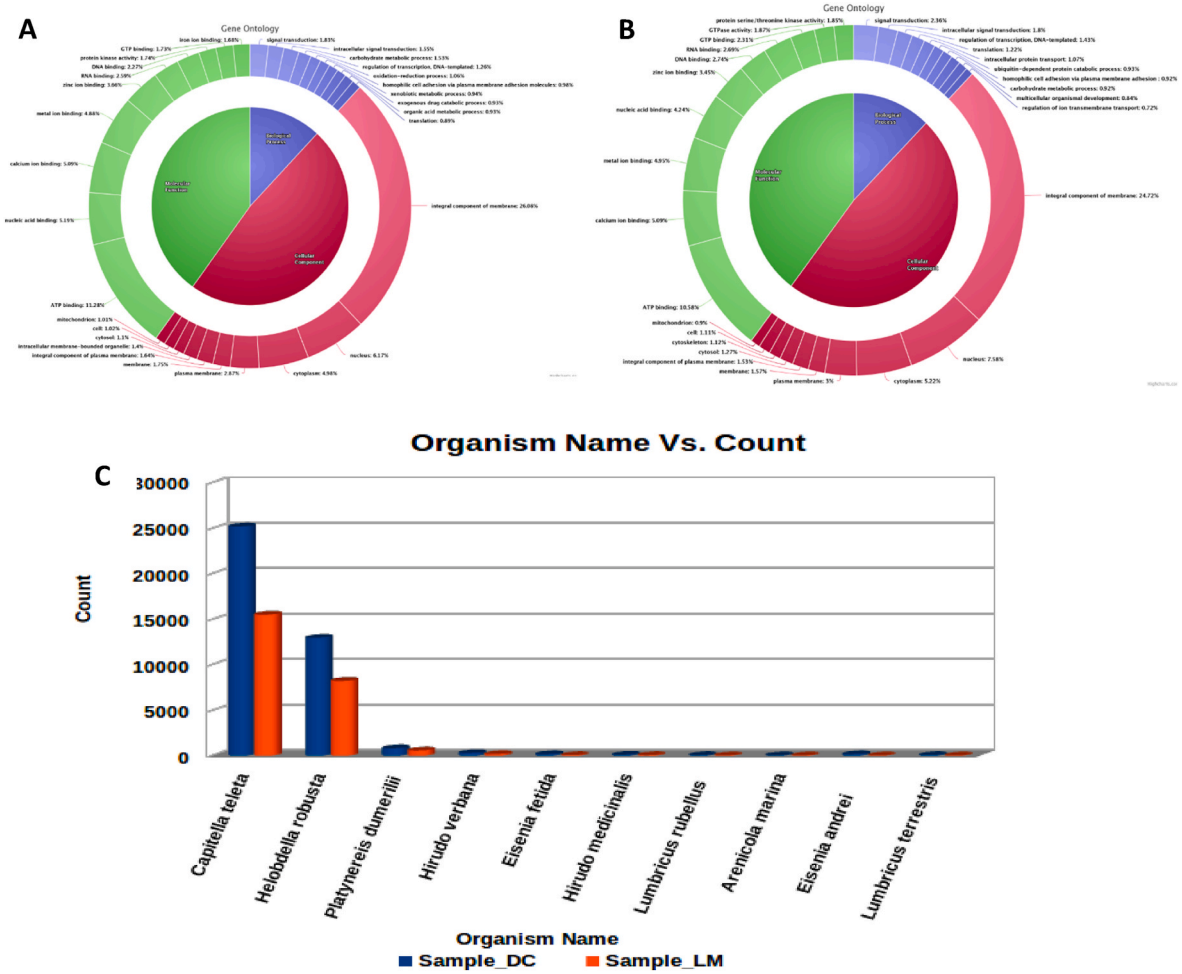

Fig. 3. A–B Gene Ontology (GO) chart of earthworms. **A** *Drawida calebi* (DC), **B** *Lampito mauritii* (LM). **C** Organism similarity chart of both (DC), and (LM) samples.

0.43% in DC sample (Fig. 3, C). Gene ontology (GO) enrichment analysis of differential gene expression (DGE) with respect to functional categories reveal several upregulated and down regulated genes in intact tissue and regenerated tissue of two earthworms *Drawida calebi* (DC) and *Lampito mauritii* (LM). With respect to comparison of molecular function category of gene ontology (GO) between DC and LM, the predominant pathways are occupied by ATP binding (11.28% vs. 10.58%), calcium binding (5.09% in both cases), metal ion binding (4.88% vs. 4.95%), nucleic acid binding (4.88% vs. 4.24%).

Similarly comparison of biological processes category of GO between DC and LM reveals predominance of signal transduction (1.83% vs. 2.36%), intracellular signal transduction (1.55% vs. 1.8%), carbohydrate metabolic process (1.53% vs. 0.92%), regulation of transcription (1.26% vs. 1.43%). On the other hand, comparison of cellular component of GO between DC and LM shows predominantly integral component of membrane (26.08% vs. 24.72%), nucleus (6.17% vs. 7.58%), cytoplasm (4.98% vs. 5.22%), plasma membrane (2.87% vs. 3%) (Fig. 3 *A and B*.

It was observed that both intact and regenerated tissue had several unique genes in earthworms, *Drawida calebi* and *Lampito mauritii*. Total number of uncharacterized proteins were 2859 in DC and 1149 in DC_RE whereas these values were 1729 in LM and 1165 in LM_RE samples.

3.6. KEGG pathway analysis

KEGG pathway of annotated genes between regenerated and intact tissue were almost found to be similar in DC and LM samples. In regenerated tissue of DC and LM samples, genes involved in pathway of membrane trafficking, protein kinases, endocytosis, TGF-β signaling, mTOR signaling, FoxO signaling, glycine, serine and threonine metabolism, autophagy and ABC transporters were found to be upregulated. On the contrary, some genes particularly involved in pathways such as *wnt* signaling, *notch* signaling in DC regenerated sample were down regulated. Genes for spliceosomes, ubiquitin mediated proteolysis, cytoskeleton proteins, exosome pathways in regenerated LM samples were found to be down regulated whereas these pathways were found to be upregulated in DC regenerated samples (Fig. 7 and 8)(supplementary file).

4. Discussion

Earthworms exhibits remarkable regeneration of lost body parts that is exhibited by dedifferentiation and cell activation similar to regeneration of vertebrates, making earthworms more suitable than hydra or planarians as an experimental material [16]. The present study is the first study of transcriptome profile of regenerating tail in two different genera of earthworms, *Drawida calebi* (Gates, 1945) and *Lampito mauritii* (Kinberg, 1867), where about 2859 transcripts in DC, 1149 transcripts in DC_RE samples, 1729 transcripts in LM and 1165 transcripts in LM_RE samples were found to be uncharacterized, without having any homologs with any known species, which is quite high compared to uncharacterized 315 transcripts observed in earthworm, *Eisenia foetida* during regeneration [6]. This implies involvement of more number of uniquely expressed genes in *Lampito* and *Drawida* during regeneration process.

During regeneration of tail, up-regulation of genes such as *beta catenin*, *Sox, notch, FGF* along with *frizzled, early growth response (EGR) protein, PI-3 kinase* genes that induce tail formation were observed in both DC_RE and LM_RE samples. In fact wound created at the site of tail amputation during study of regeneration, activates *wnt* and *beta catenin-1* genes which inhibits the gene, *notum* to promote tail regeneration and stop regeneration of head. This was evidenced by down regulation of genes, *labial* and *notum* that promote head formation in planarians, and earthworms [4]. The differential expression of these genes were also observed during regeneration of earthworm *Eisenia foetida* and planaria *Dugesia japonica* observed by various workers [6,17–19]. These genes were found to be involved in synthesis of extracellular matrix, suppression of immune response, activation of genes required for differentiation and mitogenesis, acceleration of cell growth, proliferation and differentiation of stem cells. However expression of many up-regulated genes such as *Oct4*, *nanog*, *Lin28, cmyc, MCM, Rap1, fascin, BMP*, *even skipped, brachyury* during regeneration of *Eisenia* and *Dugesia* reported by different workers were not observed in the present study indicating that either species specific gene expression was playing an important role in regenerating the lost tissue or other alternative genes may be playing role in regeneration of tail which need to be verified by different confirmatory molecular experimental approaches [4,6,18]. During regeneration of tail in both DE_RE and LM_RE samples, Wnt-signaling components encoding *Wnt1, Dishevelled*, transcription factors *hox4, lox2, post2* were up-regulated along with induction of transcription of early growth response genes. Besides, expression of genes such as *slit* known for ventral surface formation, *netrin* gene for axon guidance and cell migration during development of regenerated tissue observed in planarians and earthworm *Eisenia andrei* were also found to be similarly upregulated in both DE_RE and LM_RE samples [4, 20]. In both DC_RE and LM_RE samples, up-regulation of gene *run* was observed which promotes gut formation in *Caenorhabditis elegans* and heterogeneity in neoblasts near wounds in planarians and earthworm, *Eisenia andrei* [4,20,21].

Comparison of differential expression of genes between intact and regenerated samples show up-regulation of specific genes meant for promoting regeneration and immunity. In DC_RE samples, there was up-regulation of Na,K-ATPase activity that plays an important role in regulating both the structure and function of polarized epithelial cells during regeneration in newts, planarians [13,20]. There was up-regulation of *notch* protein expression to carry out cell-cell signaling needed for self renewal in regenerated tissue [22]. The up-regulation of gene *for gycine cleavage system* for catabolism of glycine was meant for controlling the stem cell pluripotency needed for repair of injured tissue during regeneration [23]. There was higher expression of *caudal protein* for formation of posterior portion during segmentation of body [24].

Many genes are found to be exclusively expressed in particular genus during regeneration of animals. For instance, based on the study of mouse and zebrafish models during regeneration, up-regulation of *Katanin p80 subunit B1* gene (promote neurogenesis), *Dynamitin* gene (neuron transmission), *Adaptor Protein 2* (formation of contact between neurons in newly regenerating tissue), *Neurexin gene* (cell adhesion between neurons), *Rer1* (regulating neural stem cell maintenance) were observed during regeneration in LM_RE sample, [25–29]. Similar to unique genes up-regulated in LM_RE sample, many genes were exclusively up-regulated in DC_RE. In DC_RE, there were up-regulation of genes *semaphorin* (promote neuronal regeneration and immune response) [30], *magi* (regulate somite segmentation and neurogenesis) [31], *Rho GTPase* (promotes wound enclosure through oxidative signaling) [32]. The functions of these genes were observed in zebrafish, *Drosphila* and mice models.

Thus majority of genes in DC_RE and LM_RE samples were dedicated to immune response, maintenance of cytoskeleton, resisting oxidative stress and neuron regeneration for cell cell communication.

The findings of KEGG pathway analysis of annotated differential gene expression (DGE) in the present study were similarl to that observed in earthworm, *Eisenia fetida* during regeneration experiment conducted by Yang and coworkers (2019) [33], indicating that these pathways were essential to begin cellular and metabolic processes, signal transduction for regeneration. The peculiar observation on down regulation instead of upregulation of expression of spliceosomes, ubiquitin mediated proteolysis, cytoskeleton proteins, exosome pathways in regenerated LM samples, could be due to lack of inclusion of at least triplicate samples for analysis of DGE in LM sample. However this problem has not arised in DC regenerated tissue and the different KEGG pathways of DC are similarly observed during regeneration of planarians and earthworm, *Eisenia*. Injury stress during regeneration has created proapoptotic as well as activation of antioxidant pathways via FoxO

signaling; promotion of cell cell adhesion, differentiation of cells via TGF-β signaling; transport of morphogens for body pattern formation via active formation of exosome [35–37].

The report on organism similarity in present study show significant differences from earlier studies on earthworms, *Eisenia fetida* and *Dendrobaena octaedra* [34], which may be due to different lineages between current studied samples DC, LM and *Eisenia* or *Dendrobaena* that contributed to differences in transcripts.

5. Conclusion

Both earthworms, *Drawida calebi* and *Lampito mauritii* were found to show up regulation of genes such as *beta catenin, Sox, notch, FGF, frizzled, early growth response (EGR)* during tail regeneration. However, many genes involved in tail regeneration of earthworm *Eisenia fetida* and planarians were not observed in the present study which indicates species specific expression. Majority of genes in *Drawida* and *Lampito* were dedicated to immune response, maintenance of cytoskeleton, resisting oxidative stress and promoting neuronal regeneration for cell-cell communication during tail regeneration. These two earthworm genera were having less similarity with the transcripts of well studied earthworms, *Eisenia fetida* and *Dendrobena octaedra*. Analysis of annotated differentially expressed genes reveal that these genes were enormously concentrated in metabolism, cellular process and environmental information processing.

Contribution

Concept design, analysis, paper writing by SSN, experiment set up by RS, identification of species, paper writing, proofreading by SKS.

Declaration of competing interest

We the authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of ineterest

We do not have any conflicts of interest.

Acknowledgments

We acknowledge the financial support from SCIENCE & ENGINEERING RESEARCH BOARD (SERB), Dept. of Science and Technology, Govt. of India (**letter no. FILE NO. EEO/2016/000611**). Due acknowledgement is extended to Center of Excellence (RUSA), Utkal University for academic support. We extend our sincere thanks to Head of the Dept. of Zoology, Utkal University for providing lab facility to carry out this work. We are grateful to Genotypic Technology (P) Ltd., #2/13, Balaji Complex, 80 feet road, R.M.V. 2nd Stage, Bangalore-560094, India for carrying out RNA sequencing and analysis using Illumina NGS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.101092.

References

[1] A.S. Mehta, A. Singh, Insights into regeneration tool box: an animal model approach, Dev. Biol. 453 (2019) 111–129, https://doi.org/10.1016/j.ydbio.2019.04.006.
[2] J. Tao, Q. Han, H. Zhou, X. Diao, Transcriptomic responses of regenerating earthworms (*Eisenia foetida*) to retinoic acid reveals the role of pluripotency genes, Chemosphere 226 (2019) 47–59.
[3] M. Li, M. Wang, W. Wang, L. Wang, Z. Liu, J. Sun, K. Wang, L. Song, The immunomodulatory function of invertebrate specific neuropeptide FMRFamide in oyster *Crassostrea gigas*, Fish Shellfish Immunol. 88 (2019) 480–488.
[4] P.W. Reddien, The cellular and molecular basis for planarian regeneration, Cell 175 (2018), https://doi.org/10.1016/j.cell.2018.09.021, 4,10.
[5] M.L. Scimone, L.E. Cote, P.W. Reddien, Orthogonal muscle fibres have different instructive roles in planarian regeneration, Nature 551 (2017) 623–628.
[6] A. Bhambri, N. Dhaunta, S.S. Patel, M. Hardikar, A. Bhatt, N. Srikakulum, S. Shridhar, S. Vellarikkal, S. Vellarikal, R. Pandey, et al., Large scle changes in the transcrome of *Eisenia fetida* during regeneration, PloS One 13 (9) (2018), e0204234, https://doi.org/10.1371/journal.pone.0204234.
[7] S. Paul, L.H. Heckmann, J.G. Sørensen, M. Holmstrup, A. Arumugaperumal, S. Sudhakar, Transcriptome sequencing, de novo assembly and annotation of the freeze tolerant earthworm, Dendrobaena octaedra, Genrep, 2018, https://doi.org/10.1016/j.genrep.2018.10.010.
[8] J.M. Julka, B.K. Senapati, Earthworms (Oligochaeta, annelida) of Orissa, India, in: Records of Zoological Survey of India, Zoological Survey of India, 1987.
[9] M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet. journal 17 (1) (2011) 10–12, https://doi.org/10.14806/ej.17.1.200. May2011.
[10] A. Bankevich, S. Nurk, D. Antipov, A.A. Gurevich, M. Dvorkin, A.S. Kulikov, V. M. Lesin, S.I. Nikolenko, S. Pham, A.D. Prjibelski, et al., SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing, J. Comput. Biol. 19 (2012) 5, https://doi.org/10.1089/cmb.2012.0021.
[11] L. Fu, B. Niu, Z. Zhu, S. Wu, W. Li, Cd-Hit, Accelerated for clustering the next-generation sequencing data, Bioinformatics 28 (23) (2012) 3150–3152, https://doi.org/10.1093/bioinformatics/bts565.
[12] B. Langmead, S.L. Salzberg, Fast gapped-read alignment with Bowtie 2, Nat. Methods 9 (4) (2012) 357–359, https://doi.org/10.1038/nmeth.1923.
[13] S. Anders, W. Huber, Differential expression analysis for sequence count data, Genome Biol. 11 (10) (2010) R106, https://doi.org/10.1186/gb-2010-11-10-r106.
[14] Y. Moriya, M. Itoh, S. Okuda, A.C. Yoshizawa, M. Kanehisa, KAAS: an automatic genome annotation and pathway reconstruction server, Nucleic Acids Res. 35 (2007) W182–W185, https://doi.org/10.1093/nar/gkm321.
[15] MISA. http://pgrc.ipk-gatersleben.de/misa/.
[16] N. Xiao, F. Ge, C.A. Edwards, The regeneration capacity of an earthworm, Eisenia fetida, in relation to the site of amputation along the body, Acta Ecol. Sin. 31 (2011) 197–204.
[17] Y.F. Qin, H.M. Fang, Q.N. Tian, Z.X. Bao, P. Lu, J.M. Zhao, et al., Transcriptome profiling and digital gene expression by deep sequencing in normal/regenerative tissues of planarian *Dugesia japonica*, Genomics 97 (2011) 364–371.
[18] P. Zheng, Q. Shao, X. Diao, Z. Li, Q. Han, Expression of Stem Cell Pluripotency Factors during Regeneration in Earthworm *Eisenia Foetida*, Gene, 2015, https://doi.org/10.1016/j.gene.2015.08.034.
[19] J. Yuan, Z. Wang, D. Zou, Q. Peng, R. Peng, F. Zou, Expression profiling of planarians shed light on a dual role of programmed cell death during the regeneration, J. of cellular biochemistry (2018), https://doi.org/10.1002/jcb.20779.
[20] Y. Shao, X.B. Wang, J.J. Zhang, M.L. Li, S.S. Wu, X.Y. Ma, X. Wang, H.F. Zhao, Y. Li, H.H. Zhu, D.M. Irwin, et al., Genome and single-cell RNA- equencing of the earthworm Eisenia Andrei identifies cellular mechanisms underlying regeneration, Nat. Commun. 11 (2020) 2656, https://doi.org/10.1038/s41467-020-16454-8.
[21] S. Nam, Y.H. Jin, Q.L. Li, K.Y. Lee, G.B. Jeong, Y. Ito, et al., Expression pattern, regulation, and biological role of runt domain transcription factor, run, in *Caenorhabditis elegans*, Molecular and Cellular Biology, Jan. 22 (2) (2002) 547–554, https://doi.org/10.1128/MCB.22.2.547–554.2002.
[22] C. Sato, G. Zhao, M.X.G. Ilagan, An overview of notch signaling in adult tissue renewal and maintenance, Curr. Alzheimer Res. 9 (2) (2012) 227–240, 2.
[23] S. Tian, J. Feng, Y. Cao, S. Shen, Y. Cai, D. Yang, R. Yan, L. Wang, H. Zhang, S. Zhong, et al., Glycine cleavage system determines the fate of pluripotent stem cells via the regulation of senescence and epigenetic modifications, Life Science Alliance 2 (5) (2019), e201900413, https://doi.org/10.26508/lsa.2019004.
[24] H. Shir-Shapira, J. Sharabany, M. Filderman, D. Ideses, A. Ovadia-Shochat, M. Mannervik, Juven Gershon, Structure-function analysis of the Drosophila melanogaster caudal transcription factor provides insights into core promoter-preferential activation, J. Biol. Chem. 290 (28) (2015) 17293–17305, https://doi.org/10.1074/jbc.M114.632109.
[25] W.F. Hu, O. Pomp, T. Ben-Omran, A. Kodani, K. Henke, G.H. Mochida, T.W. Yu, M. B. Woodworth, C. Bonnard, Katanin p80 regulates human cortical development by limiting centriole and cilia number, Neuron 84 (2014) 1240–1257, https://doi.org/10.1016/j.neuron.2014.12.017.
[26] H. LaMonte Bernadette, K.E. Wallace, B.A. Holloway, O.J. Ascan, M. Tokito, T. V. Winkle, D.S. Howland, E.L.F. Holzbaur, Disruption of dynein/dynactin inhibits axonal transport in motor neurons causing late-onset progressive degeneration, Neuron 34 (2002) 715–727.
[27] J.W. Kyung, I.H. Cho, S. Lee, W.K. Song, T.A. Ryan, M.B. Hoppa, S.H. Kim, Adaptor Protein 2 (AP-2) complex is essential for functional axogenesis in hippocampal neurons, Sci. Rep. 7 (2017) 41620, https://doi.org/10.1038/srep41620.
[28] C. Dean, T. Dresbach, Neuroligins and neurexins: linking cell adhesion, synapse formation and cognitive function, TRENDS in Neurosciences 29 (2006). http://www.bioinformatics.babraham.ac.uk/projects/fastqc/. (Accessed 1 January 2006).
[29] T. Hara, I. Maejima, T. Akuzawa, R. Hirai, H. Kobayashi, S. Tsukamoto, M. Tsunoda, A. Ono, S. Yamakoshi, S. Oikawa, et al., Rer1-mediated quality control system is required for neural stem cell maintenance during cerebral cortex

development, PLoS Genet. 14 (9) (2018), e1007647, https://doi.org/10.1371/journal.pgen.1007647.
[30] Y. Goshima, Y. Sasaki, T. Nakayama, T. Ito, T. Kimura, Functions of semaphorins in axon guidance and neuronal regeneration, J. Pharmacol 82 (2000) 273–279.
[31] G.J. Wright, J.D. Leslie, L. Ariza-McNaughton, J. Lewis, Delta proteins and MAGI proteins: an interaction of Notch ligands with intracellular scaffolding molecules and its significance for zebrafish development, Development 131 (22) (2004) 5659–5669, https://doi.org/10.1242/dev.01417.
[32] H. Fu, H. Zhou, X. Yu, J. Xu, J. Zhou, X. Meng, J. Zhao, Yu Zhou, A.D. Chisholm, S. Xu, Wounding triggers MIRO-1 dependent mitochondrial fragmentation that accelerates epidermal wound closure through oxidative signaling, Nat. Commun. (2020), https://doi.org/10.1038/s41467-020-14885-x.
[33] Y. Yang, Y. Sun, N. Zhang, J. Li, C. Zhang, X. Duan, Y. Ding, R. Zhao, Z. Zheng, D. Geng, Y. Sun, The up-regulation of two identified wound healing specific proteins-HSP70 and lysozyme in regenerated *Eisenia fetida* through transcriptome analysis, J. Ethnopharmacol. 237 (2019) 64–73, https://doi.org/10.1016/j.jep.2019.03.047.
[34] V. Ponesakki, S. Paul, D.K.S. Mani, V. Rajendiran, P. Kanniah, S. Sivasubramaniam, Annotation of nerve cord transcriptome in earthworm, *Eisenia fetida*, Genomics Data 14 (2017) 91–105.
[35] Z. Tothova, D. Gilliland, Gary FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system, Cell Stem Cell 1 (2007) August, https://doi.org/10.1016/j.stem.2007.07.017.
[36] -A. Kwaka Eun, Y. Lee Nam, Synergetic roles of TGF-β signaling in tissue engineering, Cytokine 115 (2019) 60–63, https://doi.org/10.1016/j.cyto.2018.12.010.
[37] J. Basu, J.W. Ludlow, Exosomes for repair, regeneration and rejuvenation, Expet Opin. Biol. Ther. (2015), https://doi.org/10.1517/14712598.2016.1131976.