ABSTRACT

Eisenia fetida, which crawls through and characteristically consumes its own home/habitat, is dependent on microbial associations for its growth and reproduction. It devours microbe-rich compost and animal manures, thus constantly exposing itself to pathogens. It hosts a good number of bacteria in its gut and coelom from its habitat. The gut contains more (many times) live bacteria than coelom. A certain fraction of the innumerable diverse bacteria present in the earthworm feed, during its passage through the gut, enjoys a selective advantage in the gut environment to contribute transformation of the soil biogeochemistry. As members of *Firmicutes* constituted the major fraction of the cultivable bacterial diversity, we focused our study on *Eisenia-Firmicute* association more specifically *E. fetida-Bacillus* spp. association. In the present study, the interrelationship between habitat (processed cow dung) and the earthworm (*E. fetida*) has been studied in the laboratory mimicking the *in-situ* conditions. The undigested residue of consumed food material excreted by the herbivorous cows, defined as cow dung, contains high titres of culturable *Bacillus* spp. ($> 10^{13}$ g^{-1} processed cow dung) throughout the period of processing until it gets suitable for feeding the earthworms. Since several *Bacillus* species present in the gut of *E. fetida* play a major role in the degradation of polymeric materials, they are predominant with titres $> 10^{11}$ g^{-1} gut content. In this study, twenty bacterial strains from the different regions of gut of *E. fetida* were isolated followed by molecular characterization by 16S rRNA genes. Most of the strains appear to be novel at the species level and at least one at the genus level. Further biochemical tests and chemotaxonomic studies were carried out for the bacterial strains ET03^T, EPG1^T, EAG2^T and EAG3^T to confirm the molecular findings.

Community composition, functional and metabolic dynamics were assessed by metagenomic analyses. Whole metagenome sequences were derived from experiments carried out on Illumina MiSeq platform. Taxonomic hit distribution at phylum level shows that the metagenome has 28.5% *Proteobacteria*, 15.2 % *Firmicute*s, 13.1% *Actinobacteria* and 13% *Bacteroidetes*. This finding has validated the culture-dependent data; where we found *Firmicutes* (~40%), *Proteobacteria* (~30%) and *Actinobacteria* (~30%); data on *Bacteroidetes*, being anaerobic, could not be found in the study (culture-dependent)as it was limited to mainly the aerobic and

microaerophilic cultivable bacteria. Whole Genome Sequences (WGS) of the unique *Eisenia*-associated-bacterial strains, ET03^T, EPG1^T, EAG2^T and EAG3^T, have been derived from Illumina NextSeq 500 NGS platform.

Coelome microbiome of *E. fetida* was explored by both culture-dependent and independent methods. Coupled with this aspect was the study related to the function of the host coelomocytes. For the culture-dependent exploration of bacterial diversity, we aseptically harvested the coelomic fluid (CF) in sterile capillary tubes. Serially diluted CF was spread on Luria Agar (LA) plates for heterotrophic bacterial growth and Hichrome Bacillus Agar (HBA) plates for enumeration of *Bacillus* specific population load in CF. Colonies from HBA plates were picked, purified and identified by means of 16S rRNA gene phylogeny. To estimate culture independent bacterial diversity, CF was used as the template for PCR amplification of 16S rRNA genes. The amplicons were cloned and randomly selected for restriction digestion using *Hae* III endonuclease for RFLP analysis. A UPGMA tree was generated from the restriction map to reveal the diversity of 16S rRNA gene sequences. The clusters obtained from UPGMA tree were cross-validated with 16S rRNA sequences derived from pure cultures resulting from culture-dependent experiments.

Coelomic space of the composter earthworm *E. fetida* is not sterile. Three *Bacillus* spp. viz. *Bacillus megaterium, B. cereus, B. pumilus* have been prominently observed at varied concentrations in the coelomic fluid of *Eisenia* during various stages of vermicomposting at regulated laboratory conditions. *B. coagulans* remained in the processed raw cow dung (PrCD) as the most preferred group (10^7-10^8 cfu/g PrCD) but was absent in the coelomic space of *E. fetida*. *B. megaterium* is invariably found in coelomic fluid of *Eisenia* (10^4 cfu/ml) along with variable presence of other co-species. *Bacillus thuringiensis,* which is an opportunistic invertebrate pathogen, occasionally observed in the processed raw cow dung (PrCD) and consequently in the coelomic fluid. High dose of *B. thuringiensis* inoculation in the PrCD leads to infection of coelomic fluid with the same. *E. fetida* has evolved various immune-defense mechanisms which are assigned to coelomocytes, present in it's coelomic fluid. Light microscopic studies revealed the following morphologically distinct groups of coelomocytes in *E. fetida* - amoebocytes (23±9%), granulocytes(18±7%) eleocytes(6±3%). Amoebocytes with phagocytotic activity occasionally bear inclusion bodies. Coelomic fluid contains 6×10^5/ml naturally occurring bacteria. The number

of potential phagocytic cells is >10 higher than the microbial population. Phagocytosis by coelomocytes can be modulated by humoral components and thus phagocytosis is promoted at certain states to check the pathogen spp. Forceful introduction of *B. thuringiensis* in the coelomic space of *E. fetida* increases the rate of phagocytosis.

E. fetida, also, has gained importance as a model in regeneration biology. Detailed histological shreds of evidence of tissue-level dynamics during regeneration are not available. The present study has been undertaken to describe tissue reorganization after amputation in *E. fetida*. Transverse amputation of adult *Eisenia fetida* at different regions of the body followed by survival and development studies revealed that anterior fragments can regenerate missing posterior regions when amputations are done at least beyond the clitella. Internal tissue reorganization and formation of the major tissues are complete during posterior regeneration within 11^{th} day post amputation. Histological studies reveal that neoblast cells originate from de-differentiation of the longitudinal muscle cells, basal epithelial cells and cells of visceral peritoneum. The blastemal mass comprising chloragogue tissue has also been observed. Histological study of the anterior amputees revealed gross tissue orientation deformities. The absence of de-differentiated muscle cells and no growth in visceral peritoneum are distinctly observed; blastema did not form. The population of *B. cereus* and *B. megaterium* is significantly reduced and the number of eleocytes was significantly increased during the middle stage of posterior regeneration. Finally differential gene expression in coelomocytes under bacterial challenge in comparison to control was analysed via genome-wide transcriptomics.

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