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Cellulolysis: A transient property of earthworm or symbiotic/ingested microorganisms?

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Abstract- The Cellulose is the most abundant polymer in nature and constitutes a large pool of carbon for microorganisms, the main agent responsible for soil organic matter decomposition. Cellulolysis is broughtabout by interaction of complex communities of micro-organisms namely, fungi and bacteria. Earlier studies have shown that the substrate cellulose is insoluble, therefore bacterial and fungal degradation occurs exocellularly either in association with the outer cell envelop layer or extracellularly. Earthworms play an important role in the degradation of substrate indirectly by affecting microbial population structure and dynamics and also directly simply because their gut is capable in undertaking cellulolytic activity. Thus, products of cellulose hydrolysis are available as carbon and energy sources for other microbes that inhabit environment in which cellulose is biodegraded, and this availability forms the basis of many biological interactions. Yet, there are still few questions to be answered as to whether cellulase comes from the earthworm or from symbiotic ingested microorganisms during the cellulolysis? Also, essentially nothing is known about the exo or extracellular assembly of complex cellulase symptoms of earthworm. This review, therefore, aims at providing existing information on the subject covering various aspects viz., structure of cellulose, secretion of cellulase and their activity in earthworm gut, interaction among the members of cellulose decomposing microbial communities with earthworm individual as well as in-combination and the origin of cellulase. Despite considerable literature available on the subject, still there are questions with regard to origin of cellulase either from gut or from symbiotic ingested micro-organism and the role of microorganisms in the biodegradation of cellulose that we understand take place in the gut of earthworm (Aira et al, 2006; Kumar et al, 2010). Therefore ,there is a need to further investigation to resolve these questions. Present review is an endeavor in this direction and updates the existing information to pave way for further research.

I. INTRODUCTION

Cellulose is the most abundant of all naturally occurring organic compounds, probably comprising at least a third of all the matter on the earth that enters terrestrial ecosystems, and therefore, represents a huge source of energy for micro-organisms, the main agent responsible for soil organic matter decomposition. In nature, cellulolysis occurs as a result of the combined action of fungi and bacteria with different substrates requirement that shift their biomass depending on what substrate is being metabolized (Aira et al, 2007). The biological degradation of cellulose has been of paramount importance in the activities of the living systems. Each year photosynthetic fixation of CO_2 yields more than 10^{11} tons of dry plant material worldwide (Schlesinger, 1991) and almost half of this material consists of cellulose (Eriksson et al, 1990). The size of cellulose molecules (degree of polymerization) varies from 7000 to 14000 glucose moieties per molecule in secondary walls of plants but may be as low as 500 glucose units per molecule in primary walls (Ljungdahl and Eriksson, *1985;* Richmond, *1991*).

Cellulose fibrils are embedded in a matrix of other polymers, primarily hemicellulose, pectins and proteins (Richmond, 1991). Often the most abundant hemicelluloses (xylan) have β-1, 4 linked xylopyranose backbone with attached side groups of acetate, arabinofuranose and O-methyl glucuronic acid (Biely, 1985; Eriksson et al, 1990). Due to this molecule complexity, the degradation of cellulose is a slow process and limited by several factors involving celluloses, such as concentration, location and mobility of the enzymes (Kumar et al, 2010). The type of organisms involved in cellulolysis depends on the environmental conditions. Under aerobic conditions they are mainly fungi, bacteria and actinomycetes; and under anaerobic conditions, they are mostly bacteria (Richmond, 1991).

Earthworms represent an important portion of soil invertebrate biomass, and in many ecosystems they are undoubtedly the key organisms in organic matter decomposition, modifying soil nutrients and microbial dynamics with long history of uses. Five hundred years ago, Shizhen li compiled the famous medical book *Compendium of Material*, in which earthworm (Earth dragon) was recorded as a drug prescribed for antipyretic and diuretic purposes in the form of dried powder in clinic (Pan et al, 2010). Although, microorganisms are largely responsible for organic matter decomposition, yet earthworms may also affect the rate of decomposition directly by way of feeding and digesting organic matter along with microorganisms, or indirectly decomposing through their interactions with the microorganisms. This basically involves stimulation or deactivation of the microbial populations (Aira et al, 2007). Not surprisingly, over the years a wide range of equally varied cellulose degrading microbial communities have evolved. This article covers earthworms and microbes that have been studied most extensively in recent years, particularly focusing on those environments that contribute significantly to the cycling of carbon on global scale. The information included are: Physiology of cellulolytic microorganisms; the enzyme system produced by worms/micro-organisms and; their interactions with earthworms in degradation of cellulose. In addition, the review also attempts to draw attention to related aspects, needing further study.

II. The Cellulose

Cellulose is the most abundantly produced homopolymer in terrestrial environments, consisting of glucose units joined by β–1, 4 bonds. The disaccharide cellulobiose is regarded as the repeating unit in cellulose as much as the glucose unit is rotated by 180^0 relative to its neighbor. Cellulose molecules are strongly associated through inter and intra molecular hydrogen-bonding and Vander Waals forces that result in the formation of microfibrils, which in turn form fibers. Cellulose molecules are oriented in parallel, with reducing ends of adjacent glucon chains located at the same end of a microfibril. These molecules form highly ordered crystalline domains interspersed by more disordered amorphous regions. The degree of crystallinity in native cellulose is 60-90% (Leschine, 1995). Cellulose can take on at least four different crystalline forms as determined by X-ray crystallography. The predominant and native form is referred to as cellulose I. The percentage and the crystalline form of cellulose depend on cell type and development stage. Cellulose almost never occurs alone in nature but is usually associated with other plant substances. This association may affect its natural degradation. Cellulose imparts tensile strength to the wall to resist turgor pressure. High compression strengths achieve the wall to resist turgor pressure when lignin replaces water in the matrix of cell walls. Lignification greatly increase bonding within the wall and produces rigid, woody tissues able to withstand the compressive force of gravity (Richmond, 1991). The hemicelluloses surround the cellulose microfibrils and occupy spaces between fibrils (Eriksson et al,1990).This polymer must be degraded, at least in part, before cellulose in plant cell walls can be effectively degraded by cellulolytic bacteria (Sinner et al,1976, 1979) and earthworms (Nozaki, 2010). Moreover, arabinofuranosyl groups may be esterified by aromatic acids such as ferulic and þ-coumaric acid (Hartley and Ford, *1989*) and may participate in lignin hemicellulose cross-linkages (Scalbert et al, 1985), further complicating the degradation.

III. Cellulolytic enzymes

Enzymatic activities have been widely used as an index of soil fertility or ecosystem status because they are involved in biological transformation of native and foreign compounds in soils (Tate, 2000). However, the enzymology of cellulose degradation has been an area of active research for more than 40 years. Over the years,the focus of this research has frequently shifted. For example, the initial thrust to understand the mechanism of cellulose degradation was spurred by a fungal attack on the cotton clothing and tents of troops stationed in Southeast Asia during World War II. Research directed towards developing ways to inhibit fungal celluloses was carried out at the US Army Research and Development command in Natick, Massachusetts. Under the direction of Reese and Mandels, this work ultimately led to the development of seminal concepts related to the mechanism of cellulose degradation, including the role of synergism among components of the cellulase system (Gilligan and Reese, 1954; Reese and Mandels, 1971; Reese et al, 1950). With the energy crises, the focus of research on cellulose degradation shifted to developing systems and procedures to use cellulose and other polymers as a source of fuels and chemicals that could serve as a potential replacement for fossil hydrocarbons. The voluminous work that followed, aided by the development of recombinant DNA and nucleic acid sequencing techniques, has resulted in our current understanding of the enzyme systems produced by diverse cellulolytic organisms.

Lattaud et al. (1999) compared the origin and activities of cellulolytic enzymes present in the gut contents of some geophogous adult earthworms and demonstrated the mutualistic relationships with ingested micro-organism to cellulose. Zhang et al. (1993), Lattaud et al. (1997) and Lattaud et al. (1998) demonstrated the presence of complete cellulolytic enzymatic activities in the gut contents of geophagous adult earthworms. These enzymes allowed to degrade root and fungal substrates available in soils and this corroborates the observation that endogeic earthworms feed on litter debris and soils poor in organic matter. Glycolytic activities were evaluated both in the cultured gut wall tissues and in the culture media in order to compare the origin and activities of these enzymes and to determine whether they were released by the worms themselves or by the ingested microflora. Further, it has also been demonstrated that *Pontoscolex corethrurus* (Swift et al, 1979) from Palma sola, Veracruz (Mexico) and *Millsonia anomala* (Ladd and Butler, 1972) from Lamto (Côte d' Ivoire) require ingested microflora in order to degrade some substrates, such as cellulose and mannan (Zhang et al, 1993; Lattaud et al, 1997), in contrast to *Polypheretima elongata* (Michelsen, 1892) from sainte Anne (Martinique) which can synthesize intracellularly by themselves or extra and intracellularly enzymes of gut micro flora (Lattaud et al, 1997). Garcia et al. (1994, 1995), Benitez et al. (2002, 2005) measured several enzymatic activities in decomposition of organic matter in two microbial driven processes, namely, composting and vermicomposting. However, Tate (2000) observed no correlation between cellulase activities and microbial biomass, but (Ceccanti and Garcia (1994), Alef and Nannipieri (1995) and Nannipieri et al, *(2002)* have stated that the enzymatic activities are due to enzymes which may be in a living or dead cell, cell debris, free enzymes and/or enzymes in clay or immobilized in humic acid complex, not on microbial biomass of earthworm gut. The studies of Garvin et al. (2000) and Zhang et al. (1993) have shown that cellulase cannot be produced in the gut of *Hormogaster elisae* and *Pontoscolex Corethrarus* to hydrolyze the cellulose since, these earthworms possess a '*mutualistic earthworm microorganism digestive system*' (Trigo et al,1999). On the other hand, *Polypheretima elongata* has been shown to have a potential to synthesize cellulase (Lattaud, 1998).

IV. Cellulase activity in earthworm gut

Nozaki et al. (2009) studied the presence of endo-β-1, 4-glucanase, EC3.2.1.4 cellulase in the gut of *Pheretima*(*Metaphire*) *hilgendorfi* and detected novel cellulase gene (phhEg) from the gut of this worm and concluded that the earthworms themselves have the capacity to produce the endogenous and functional cellulase around the mid- foregut, and later use this cellulose for their cellulose digestion with the support of intestinal caecum. *Nozaki* et al. (2008) reported the amino-acid sequence of phhEG comprising 1606 nucleotides

including poly (A^+) tail and contained a single ORF, encoding a protein of 449 amino acid. Thus indicating that the cellulolytic enzymes which play the functional role in degradation of cellulose in the gut of earthworm are secreted by the earthworm, and not by the symbiotic and ingested micro-organisms?

Mishra and Dash (1979) also demonstrated presence of cellulase in midgut of *Lampito mauritii* kinberg, *Octochaetona surensis* Michaelson. *Drawida calebi* Gates and *Dichogaister boaui* Michaelson. The cellulase activity was also recorded by Urbasek (1990) in *Dendrobaena vejdovskyi, Denrobaena octaedra, Lumbricus castaneus, Lumbricus rubellus, Allolobophora caliginosa, Allolobophora rosea, Octolasium lacteum*. Parthasarathi and Ranganathan (2000) quantified the cellulase in pressmud and sawdust in reared *Lampito mauritii* and *Eudilus engeniae* and concluded that the cellulase activity is also influenced by the type of food. Earthworms which feed and depend on microbes, litter, and grit present in soil should contain battery of enzymes. Flack & Hartenstein (1984), Ranganathan & Parthasarathi (1999) have further demonstrated that earthworms predate microbes as a source of their food. During their passage through the gut, microflora populations get enhanced which might be responsible to the increased cellulase activity in gut of earthworms. The significant correlation between cellulase and microflora of earthworm gut was recorded by Kumar et al. (2010).

Discrepancies with theories based on symbiotic cellulose digestion arose from apparent contradictions between cellulolytic activity and the locations or absence of symbiotes as enzyme sources (*Strasdine and Whitaker, 1963*). They reported that cellulase activity in the digestive juice was significantly increased in proportion to body weight of snail and the total protein content of the hepato -pancreas, however, not in relation to the increases in digestive juice, bacterial counts; and therefore concluded that these enzymes were produced endogenously. Similar arguments have also been put forward by other researchers based on contradictions observed between the presence or absence of cellulolytic activities and gut microorganisms in earthworms (Tracey,1951; Needham et al, 1957; Parle,1963; Marialigetik,1979; Mishra and Dash,1980; Urbasek,1990; Urbasek and Pizl,1991; Vinceslas-Apka and Loquet, 1996).

Few studies on glycolytic enzymatic activities present in the gut contents of geophagous adult earthworms have demonstrated that these worms possess a complete cellulolytic system (Zhang et al,1993; Lattaud et al, 1997; Lattaud et al,1998) and these enzymes allow them to degrade root and fungal substrates available in soils corroborating that endogeic earthworms feed on litter debris and soils poor in organic matter. However, among the cellulolytic activities studied in *Pontoscolex corethrurus*, *Millsonia* sp. and *Lampito* sp. of earthworms, cellulolysis has been observed without any correlation to the cellulobiasic activities (Zhang, 1993).

There are also few reports of cellulolytic activity in the gut of some epigeic earthworms (Urbasek and Pizl, 1991; Lattaud et al, 1997; Zhang et al, 1993; Zhang et al, 2000). They recorded their ability to digest cellulose, although the effect exerted by earthworms on cellulolysis lie fundamentally in their interactions with micro organisms. These interactions however are the subject of a certain amount of controversies, mainly because of the variety of species, substrates and experimental conditions assayed. It is generally agreed that microorganisms, especially fungi are part of the diet of earthworms (Dominguez, 2004). Moreover, earthworms have been shown to graze selectively on fungal species (Moody et al, 1995). Schonholzer et al. (1999) reported that earthworms digest fungi & bacteria but there is an increase in the number of microorganisms during gut transit? This needed to be explained.

Recent experiments on cellulolytic activities in the gut of *Eisenia fetida* Andrei have clearly demonstrated that this species possess a complete cellulasic system for hydrolyzing cellulose (Hu et al, 2010). It is able to release by itself cellulose, whereas, a cellulase specific activity was detected in the cultured foregut, midgut and hindgut wall tissues and culture media; on the contrary cellulobiase was secreted by ingested soil microflora. The enzymatic system for degrading cellulose was species dependent and synergy between microflora and earthworm *Metaphire anomala* and *Haplochaetela elisae* have been reported (Lattaud et al, 1999). The strong mutualistic relationship was reported with *Metaphire* sp. along with the ingested soil micro organisms for hydrolyzing cellulose. Lattaud et al. (1997) recorded the cellulase produced by ingested microflora is able to degrade cellulose to cellobiose. The released cellobiase by the earthworm itself breaks down cellobiose into D-glucose. Absence of cellulase and cellobiase activities in gut wall tissues and culture media of *Pentascolex corethrurus* indicates that these cellulolytic enzymatic activities found in the gut are of microbial origin. Glycolytic enzymes produced by *Pheretima elongata*, *Drawida terrae* and *Haplochetela africanus* possess a complete enzymatic system to hydrolyse cellulose. These glycolytic enzymes were cultured from gut wall tissues in culture media which indicates that they are extracellular enzymes. Some enzymes were released in the medium only after a latent period of culture and their secretion was perhaps induced. It is worth noticing that *P. elongata* can synthesize mannanse by itself, contrary to *P. corethrurus* and *M. anomala* which requires the microflora of the soil ingested to hydrolyse mannan.

However, the origin of cellulase production, whether it is by the earthworm or by the microorganism, has not been established at the molecular level thus far; even the presence of cellulose gene (endo B-1, 4-glucanase (EGase, EC 3.2.1.4) suggests that cellulolytic enzymes which functionally degrade cellulose are secreted by earthworms themselves (*Nozka, 2010*).

V. Cellulolytic Microflora in Earthworm Gut

A large amount of information on the beneficial activities of earthworms has accumulated. The rich chemical composition of casts as well as the burrowing and ploughing action of worms is well known, but there are very few studies available on the microflora in the intestinal tract of earthworms. Louis Pasteur isolated *Bacillus* from the intestine of the earthworm (Cowan, 1951). Basslik (1913) isolated more than 50 species of bacteria from the alimentary canal of earthworm, *Lumbricus terrestris.* He found no difference between the types of bacteria isolated from the gizzard, intestine and casts and those present and collected from the soil. Bassalik (1913) also reported the isolation of a red-pigmented oxalate decomposing organism *Bacillus extorquens* from the excreta of an earthworm which had

ingested plant material containing calcium oxalate. Aichaberger (1914) observed that no diatoms, blue-green algae, desmids, yeasts or rhizopods were found alive in the alimentary canal of the worm. Jensen (1931) and Macfarlane (1952) considered earthworms as disseminating agents of spores of soil fungi like *Fusarium* and described the intestine of the worm as a breeding place for bacteria. Kelkar (1917) reported that earthworms play a part in spreading bacteria in deeper levels in the soil. According to Auel (1929) bacteria are responsible for the degradation of urea in the intestinal tract of earthworms. Hanstrom (1928) found a larger number of microorganisms present in worm casts than in soil. Henricic (1934) mentions that bacteria in the soil are brought to the surface in casts and visualized the possibility of the spread of the tetanus (*Bacillus sp.)*. Hotchkiss and Waksman **(**1936**)** found a microorganism resembling the mucous capsulated bacteria and the semi-colon group, to be regularly present in *Lumbricus terrestri*. Smith and Clark (1938) isolated non-spore forming gram- negative bacteria from the earthworm intestine which displayed interesting phenomenon of rotating colonies. Russell (1950) pointed out the microflora present in the earthworm gut may bring about the break down of organic matter and thus make the casts richer in plant nutrients. He further stated that the earthworm intestine may be the site for lignin oxidation and humus-formation. Swaby (1950) states that the intestinal bacteria of earthworms produce gums which convert the casts into water stable aggregates. Khambata & Bhat (1953, 1955, and 1957) have studied specific groups of bacteria in the worm intestines and isolated oxalate and cellulose decomposers. Joshi and Kelkar (1952) isolated viable thick-walled and thin walled fungal spores from worm gut and showed that the fungi were dispersed more rapidly in sterilized soil containing worms, than where worms were absent. Ruschmann (1953) isolated *Nocardia Polychromgenes, Actinomyces* species and *Strepomyces colicolor* isolated from the gut contents of earthworms. Hutchinson and Kamel (1956) isolated 17 species of viable fungi from alimentary canal and rectum of ten individuals of *Lumbricus terrestris*. Kozlovskaya and Zndannikova (1961) reported that the ratios of different groups of microorganisms in soil differed from those in casts, so that the spore forming bacteria & actinomycetes predominated in casts, and number of *Bacillus idosos* and *B. cerus* were greater in casts than in soil but *B. agglutinates* were recorded very few in number.A number of researches have shown increased proliferation of a variety of microorganism in the gut of earthworms *viz:* actinomycetes in *L. terrestris*, *A. longa* and *A. caliginosa* (Parle, 1963), fungi in *P. millardi* (Ghosh *et al*, 1989), *L. maruitii* and *E. engeniae* (Ranganathan and Parthasarathi, 1998) bacteria in *A. caliginosa* (Scheu, 1987). Pedersen and Hendrikson (1993) reported qualitative and quantitative changes in the bacterial flora of ingested food material during gut transit. Populations of *S. marcescens, E. coli, Salmonella enteritidis* and *B. cereus* var. *mycoides* in *L. terrestris* have been observed decreasing during passage through gut (McLean and Parkinsen, 2000).

Singleton et al (2001) examined the bacteria associated with the intestine and cast of the earthworm *Lumbricus rubellus* Hoffmeister by direct counts, cultivability studies 16SrRNA gene clone libraries and, fluorescent *in-situ* hybridization and recorded a significant fraction (24-47%) of the total numbers of prokaryotes which remains in the intestine after the casting and were tightly associated with the intestinal wall. Bacterial 16SrRNA gene clone libraries constructed from washed earthworm intestinal tissue suggested that the bacterial community was dominated by a few phylotypes that were either absent from or in low abundance, in the casts. Parthasarathi et al. (2007) analyzed the diversity of fungi, bacteria, yeast, actinomycetes and protozoa in the gut and casts of *Eudrilus eugeniae Lampito maruitii, Eisenia fetida and Perionyx excavatus* both qualitatively and quantitavely as influenced by different feed substrates like clay loam soil, cow dung and pressmud. Actinomycetes (*Streptomycess albus, S. somaliensis, Nocardia asteroides, N. caviae*) were not digested by any of these species of worms. Protozoa (*Amocba proteus, A. terriola*) and yeast (*Candida tropicalis, C. krusei, C. albicans*) were totally digested. Certain fungi like *Mucor plumbus, Cladosporium carrionni* and bacteria like *Pseudomonas aeruginosa, Bacterium antitratum, E. cloacae, Proteus vulgaris* were compeletly digested. Jyotsana et al, (2011) isolated twenty species of bacteria from the gut of earthworm (*Eisenia foetida*) and all the isolates were found positive for cellulase production. One isolate showing maximum activity was identified as *Lysini bacillus sphaericus* (formerly *Bacillus spharius*). Mishra et al. (2011) isolated *Bacillus sp., Pseudomonas sp, Staphylococus sp., Bacillus subtilus, Bacillus lentus, Azotobacter sp., Micrococcus sp, Tlavobacterium sp., Brevibacterium sp.* and *Thiobacillus sp.* from the tropical earthworm *Glyphodrillus tuberosus*, and recorded higher microbial load in gut section of the worm than undigested soil. Shankar et al. (2011) isolated cellulolytic bacteria from *Eudrilus engeniae* and assessed the cellulolytic activity in the microbes, isolated from the gut. The study has revealed that the bacterial community was responsible for the breakdown of cellulose and thereby, leading to decomposition of organic matter by earthworm.

VI. Cellulase gene

There are several reports on the cDNA cloning of cellulases (Aira et al, 2006; Giritch et al, 2006) but few on the genomic structure and sequences of their cellulase gene. The reports on plant-parasitic nematodes and the higher termite *N. takasagoensis* have cohered entire genomic structure of their cellulase genes. Two cellulase genes form each of two plant parasitic nematodes *G. rostochiensis* and *H. gluycines* (GR-eng I, 2492 bb and GR-eng2, 2388 bp; HG-eng I, 2151 bp and HG-eng 2, 2324 bp respectively) were isolated from recombinant phage genomic DNA libraries by probing the EGc DNAs. These four genes had seven introns at exactly corresponding positions in the catalytic domain coding regions (Xu et al, 2000). Additionally, GR, and HG eng2 had one additional intron at corresponding positions in the putative CBD coding regions. An entire Nt EG gene was isolated from the higher termite *N. takasaogenesis* by combining PCR amplification of genomic DNA with sets of primers designed from Nt EG cDNA sequence. A complete 13,005 bp gene including ten exons separated by nine introns was identified. In *R. speratus*, three introns of RsEG were identified interrupting exons at the corresponding positions and phases of NtEG (Tokuda et al, 1999). Other corresponding introns of RsEG and RsEG2 have also been identified from the genomic DNA of *R. speratus*. In addition to the presence of

introns, these animal cellulase genes maintain other qualities shared with other eukaryotic genes. They have all the TATA boxes in their upstream regions, polyadenylation and cleavage signals (GATAAA) in appropriate sites, and their exon/intron borders follow the eukaryotic GT/AG rule except for four minor cases in the nematode EG genes (GC instead of GU) at the 5' end (Tokuda et al, 1999; Yan et al, 1998).

Nozaki et al. (2008) detected the cellulasic acitivity of the extracts from the intestinal tissues and contents of *Pheretima (Metaphire) hilgendorfi* as a single band with molecular weight of 51k Da, which indicates that one major cellulose functions in degrading cellulose. The cellulase gene detected has a species specific cellulase produced by species specific symbiotic microorganisms. The full length cDNA of the earthworm *P. hilgendorfi* a cDNA library was constructed from the intestine tissue of *P. (M) hilgendorfi* and screened to find a positive clone (phhEG).

The deduced amino-acid sequence of phhEG comprised 1,606 nucleotides including poly (A^+) tail and contained a single ORF encoding a protein of 449 amino acids. The N terminal of phhEG was found to contain 18 amino acids signal peptide based on likelihood predictions (Nielsen et al, 1997), resulting in the formation of a mature enzyme comprised of 431 aminoacids with molecular weight of 47.1 kDa. Comparison of the deduced amino acid sequence with the available cellulase sequences showed that the phhEG sequence was closely related to the cellulase belonging to GHE9 of the other earthworm, *Eisenia andarei* and the Japanese Sea urchin, *Strongylocentrotus nudus* (Nishida et al, 2007). Moreover, the amino acid residues of phhEG have conserved the catalytic sites (Tomme et al, 1991; Tomme et al, 1992; Khademi et al, 2002) which are almost invariant among the glycoside hydrolase family (GHF) 9 of the Eukaryota (Davison and Blaxter, 2005). Nishida et al. (2007) concluded that the phhEG belongs to GHF9, and phhEG gene is found in the earthworm and not in the microorganisms of their gut. However, few studies on the origin of the digestive enzymes in the gut of earthworm have shown that *Hormogaster elisae* (Garvin et al, 2000) and *Pontoscolex corethrurus* (Zhang et al, 1993) cannot produce cellulase.

The historical debate concerning the presence of endogenous cellulase in earthworms may be settled by molecular evidence. There is no doubt that a cellulase is a protein that can be potentially produced in any life form, provided there is a corresponding gene encoding it. However, this conclusion does not answer the queries, '*Why do some worms have endogenous cellulases and others not*? or *Why isn't cellulase a common enzyme in animals like amylase or proteinase'*? We believe further investigations based on molecular evidence into cellulolysis can eventually answer these questions.

VII. Concluding Remark

The article has reviewed numerous earlier studies that deal with the degradation of cellulose by earthworms. Microorganisms that act as biological catalyst in degradation of cellulose are of enormous biotechnological interest for their hydrolytic enzymes. Great efforts have, therefore, been devoted over the years, studying microbial cellulose system, resulting in a fair understanding of the knowledge of enzymology of cellulose degradation. However, fundamental question remains unanswered as to wherefrom cellulase came through, and largely unknown, whether the cellulose comes from the earthworm or from symbiotic/ingested microorganisms during the cellulolysis, and therefore, it is nearly impossible to recognize similarities or differences in the molecular mechanisms employed by different systems. Also, essentially nothing is known about the exo or extracellular assembly of complex cellulase system of earthworms. The biotechnological potential of cellulase in earthworms cannot be fully realized without knowledge of the genetics, which is virtually non-existent at present, and is therefore, there is a need to improve understanding of physiology of cellulase in earthworms at different level of ecological niches. The organisms producing endogenous cellulase components can be summarized on the basis of the distribution of cellulolytic activities in the digestive process of earthworm. Nevertheless, origin of cellulase as to from earthworm gut or ingested microorganism is still in doubt? Simple measurements of cellulolytic activities in particular excreting organ, cannot discriminate authentic endogenous activities from the production by intracellular symbiotes. Immuno-histochemical observations using an antiserum raised against purified cellulolytic components can provide reasonable accuracy (Watanabe and Tokuda, 2001).

However, ideally, a combination of molecular methods would be most desirable to meet today's need to settle the debate about the origin of cellulase from the earthworms themselves or the symbiotic and/or ingested microorganisms. In addition mutualist earthworm microorganism's digestive system has the '*capacity to degrade cellulose*'. Hence, earthworm plays an important role in the nutrient cycling which is essential for environmental preservation and agricultural sustainability. It is hoped that this review will lead to better understanding of our knowledge on cellulose degradation by earthworms on open new vistas of research in this direction.

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