

Gut Microbiota from Lower Groups of Animals: An Upcoming Source for Cellulolytic Enzymes with Industrial Potentials

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Abstract: Cellulosic plant materials are a reliable source of renewable energy. Cellulose-based plant materials are now being used for bioenergy production as alternatives to fossil fuels. The traditional way of converting lignocellulosic materials to ethanol and other bioenergy is an expensive and environmentally unsafe process. Several research works have been conducted to find outsource of low-cost cellulolytic enzymes. Initially, fungal species were considered as sources of cellulolytic enzymes. Later on, several studies showed that bacterial species are a more potent source of cellulose-degrading enzymes. Phytophagous lower invertebrates are a good source of cellulolytic gut bacteria. They utilize a wide variety of plant materials as their food source. In this review, thorough literature studies have been made to explore the invertebrate groups that are novel sources of cellulolytic gut bacteria with high efficacy for enzyme production. This study also encompasses a brief description of cellulose, the activity, and cellulase enzyme application in industrial aspects.

Keywords: renewable energy; cellulose; cellulase; lower invertebrates; cellulolytic gut bacteria; microorganisms.

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1. Introduction

Lignocellulosic biomass is the most abundant biological macromolecules in nature. It can be a promising source of renewable raw materials for the production of biofuels and various chemicals[1-12]. The plant cell wall comprises 35-50% cellulose and 20-35% hemicellulose with 5-30% lignin that together provides 90% of the dry weight of plant materials[2]. These huge amounts of biomasses are ultimately disposed of as waste materials in nature. But proper processing of these lignocellulosic wastes can mitigate the environmental and energetic demand for sustainable and renewable bioenergy [3,13-16]. Recent trends have adopted cellulosic raw materials over fossil fuels [10,13,17,18] because of their several drawbacks. Brazil is the pioneered country in utilizing renewable energy and produces ethanol from sugarcane since the 1970s [13]. But the processing of these energy resources through instrument intensive, the thermochemical treatment process is very expensive [19] and needs an alternative one. The traditional way of converting lignocellulosic materials to ethanol requires acid-reliant hydrolysis and fermentation steps, which ended up with the formation of

a large amount of calcium sulfate deposited as waste materials with some adverse effects on the environment [13]. The development of environmentally safe and economically feasible technologies for cellulase production is the key requirement for successfully utilizing plant biomass as a viable and foreseeable carbon source. Enzymatic degradation by cellulase or hemicellulase is the cost-effective way to saccharify cellulose and hemicellulose, respectively [20,21] to its monomer [22] hexose and pentose residues. Other hydrolytic enzymes such as pectinase, xylanase, and ligninase also ensure a high rate of degradation of the cellulose to its monomer and a high yield of biofuels lignocellulosic plant biomass [23]. The rate and efficacy of ethanol production from these monomers solely depend on the fermentation efficiency and enzymatic activity of microorganisms. These enzymes have potential application in food processing, winery, textile and laundry industry, paper and pulp preparation, animal feed, agricultural industry, and waste management process [1,24-28]. In bioethanol's industrial production, basic yeast *Saccharomyces cerevisiae* is used as it has some unique features, including high productivity of ethanol and alcohol tolerance [6,29-32]. But the activity of *Saccharomyces cerevisiae* gradually decreases due to byproduct inhibition and thus restricts its application for industrial use [33,34]. Thus identification and isolation of impeccable microorganisms with high production efficacy, high yield of several biofuels, and resistances to inhibitors are the necessary steps for industrial production of biofuels from cellulosic raw materials [30,35]. Initially, several cellulose digestive enzymes have been isolated from several fungal species, but they have some limitations, including low specific activity, low thermal stability, and narrow pH range tolerance. That is why several bacterial species are being explored later on for isolation and screening of cellulase production [2]. Herbivorous animals and wood-feeders cannot synthesize cellulase within their body but rely on their gut bacterial community [14], which possess a repertoire for cellulase synthesis [36]. Some cellulolytic bacteria strains have also been identified from environmental sources such as agricultural wastes, composts, woody wastes etc. [37-41]. Recently isolation and identification of gut microbiota from the phytophagous animals have gained momentum due to the diverse availability of several phytophagous insects, beetles, termites that thrive through several ecological niches and feed on several leafy and woody materials. In this review, an in-depth literature study has been conducted to enlist the lower invertebrates recognized so far to harbor cellulolytic bacterial populations within their gut. The lower invertebrates with endogenous cellulolytic systems are also discussed here - this review also encloses a brief description of cellulase and its mode of action. Furthermore, biotechnological approaches for improving its activity and application in several industrial aspects have also been discussed.

2. Structure of Cellulose and Cellulase

Cellulose is a fibrous, tough, and water-insoluble substance, which gives rigidity to plant cell walls and is found in stalks, stems, trunk, and all the woody portions of the plant body. It is a tasteless, odorless, and hydrophilic substance. It is a linear and unbranched homopolysaccharide made of D-glucose unit with the chemical formula $(C_6H_{12}O_6)_n$. The number of D-glucose units can range from 10,000 – 15,000. In cellulose, glucose residues are linked by β 1-4 glycosidic bond. In nature, cellulose molecules exist in four crystalline forms (I α , I β , II, and III), which vary in physiochemical properties. The crystalline structure of cellulose comprises several cellulose fiber chains, which are interlinked by hydrogen bonds between hydroxyl groups of adjacent molecules. These hydrogen bonds and Vander Wall

forces together make robust and stable cellulose crystals. At ambient temperature, these hydrogen bonds of cellulose molecules can only be hydrolyzed by the cellulase enzyme system's synergistic action. Cellulase is a multienzyme system, which consists of three major components: 1, 4- β -endoglucanase (EC 3.2.1.4), 1,4- β -exoglucanase (EC 3.2.1.91) and β glucosidase (EC.3.2.1.21) (β -D-glucoside glucohydrolase or cellobiase) [42]. Endoglucanase causes random cleavage of β -1,4-glycosidic bonds along a cellulose chain, liberating a new end. Exoglucanase imparts an exo-attack at the reducing or non-reducing end of microcrystalline cellulose and produces glucose or cellobiose as the end product. β glucosidase is responsible for cellobiose hydrolysis, producing glucose as the end product [43](Figure.1).The synergistic and sequential action of all these three enzymes facilitates the complete hydrolysis of cellulose to glucose.

Symbiotic microorganisms within the insect gut have a significant contributions to the nutritional ecology of insects [44]. The persistent association of microorganisms in the insect digestive tract provides nutritional advantage through several physiological activities, including digestion and detoxification of specific foodstuff, synthesis of essential amino acids, vitamins, sterol, and nitrogen fixation, and production of pheromone [2,44,45]. Woodborer and plant-eating insects cannot digest their foodstuffs easily as cellulosic plant materials are very stable polymer and require enzymatic attack for degradation [44]. Partial degradation during insect chewing makes some cellulose of foodstuffs available for cellulase enzyme. Endoglucanases or CMCases from different microbial sources consist of catalytic modules of glycosyl hydrolase families (GH) 5–9, 12, 44, 45, 48, 51, and 74. Bacterial endoglucanases possess multiple catalytic modules, carbohydrate-binding modules (CBMs), and other modules, while fungal endoglucanases possess a catalytic module with or without a CBM [43]. Most of the exoglucanases are cellobiohydrolases (CBHs), which are produced in different forms by bacteria and fungi. The catalytic modules of CBHs belong to the glycosyl hydrolase family of 5, 6, 7, 9, 48, and 74 [43].

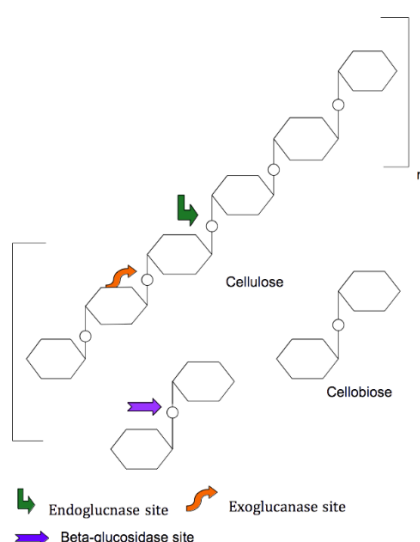


Figure 1. Cellulose hydrolysis: Activity site of endoglucanase, exoglucanase and beta-glucosidase on cellulose molecule.

The glycoside hydrolase family's exoglucanases 48 mainly act on crystalline cellulose and induce its hydrolysis, which is mediated by bacterial cellulase systems. β -glucosidase (BGs) does not possess CBM in catalytic modules and hydrolyze soluble cellodextrins and cellobiose to glucose. Cellobiose is an inhibitor of endoglucanase and CBH. Different

microorganisms produce various BGs with catalytic modules belonging to families 1, 3, and 9. Generally, aerobic fungi produce BGs extracellularly, but BGs of an anaerobic bacteria remain within their cytoplasm [43]. Microbial cellulase within the anaerobic insect gut is associated with the large enzyme integrating protein scaffoldin, which contains multiple copies of cohesin modules to integrate the different enzymes and other components. These entire components together form a multienzyme cellulosome complex [46]. Cellulase and other enzymes contain a complementary cohesin-docking domain that specifically binds to the cohesin modules of scaffoldin. Scaffoldin modules also have carbohydrate-binding domains that facilitate the cellulosome complex (Figure. 2) to bind with cellulosic substrates for degradation [46]. Cellulosome complex in association with several cellulases promotes the degradation of most recalcitrant cellulose molecules into monomeric glucose molecules utilized by insects and herbivorous animals as an energy source.

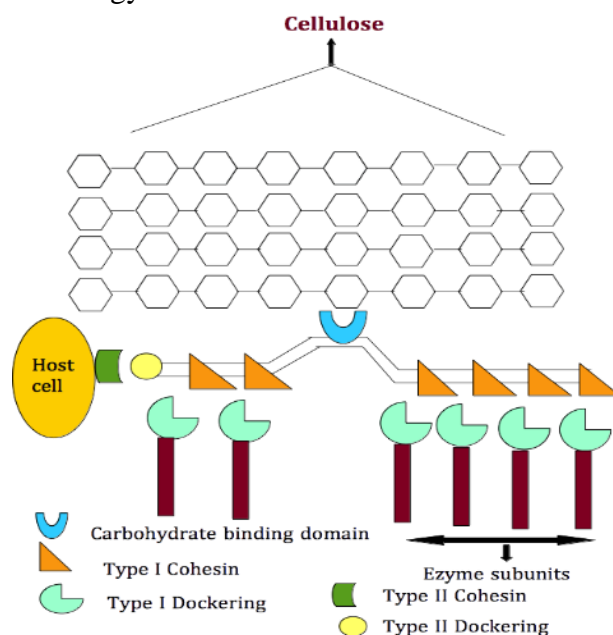


Figure 2. Mode of action of cellulase enzyme-Cellulosome structure.

3. Sources of Cellulolytic Bacteria

In recent years, an increasing trend in the search for newer sources of cellulose-degrading microorganisms is observed, keeping in view the diverse application of the cellulase in industrial sectors [1,11,24-26,28,47,48]. The fungus *Trichoderma reesei* was the most potential cellulase-producing microorganism [49] over the years. Nowadays, several studies have been aimed in search of newer microorganisms, including bacteria from several environmental sources, including municipal solid wastes [49], compost [37], agro-industrial wastes [7,35,38], soil [50-52], palm wastes (fiber and palm leaves), woody wastes, manure, straw and sugarcane molasses [53-55], mangrove soil sediment [56,57]. The aquatic environment such as moist peat and water of freshwater wetland reserve [58], lake sediments [59], water-sludge mixtures of hot-springs [16,60], and marine environment [61,62] also harbor a widespread spectrum of cellulose-degrading microorganisms.

Besides these environmental sources, many phytophagous lower invertebrates' gut microbiota has been empirically studied to obtain microorganisms with cellulolytic potential. Cellulase activity within the invertebrate digestive tract has been determined in the long past [63]. Literature reflects that the following invertebrate groups have been studied previously for cellulolytic gut bacterial source:

3.1. Arthropods-insects.

Diversified habitat and plant fiber-based diet make Arthropods a potent reservoir of several gut microbial communities. Literature survey depicts that various Arthropoda species have been explored thoroughly in search of gut microbiota with cellulolytic potential [2,64]. Among Arthropods, insects are the most studied group regarding obtaining novel gut bacterial strain, which can synthesize cellulase enzyme with industrial potential [20,44,65]. Due to the wide range of diversity and multitrophic relationships between insect groups and plant hosts, insect species harbor symbiotic bacterial communities within their digestive tract [44,66,67]. Diverse ecological niches and the phytophagous nature of insects have raised interest in studying the digestion mechanism of insect species involving microbial and endogenous cellulase [20,68]. Insect group, termites have evolved with symbiotic systems [69] that efficiently degrade lignocellulosic foodstuffs [70,71,72] and thus make the termite group a promising source of cellulolytic enzymes. Termite consists of 2000 described species that are subdivided into two groups, namely 'higher' and 'lower' group [70,73,74]. Both groups are involved in symbiotic relationships with prokaryotes, but lower groups are also the protists' host [70,73,74]. As most of the termites are wood and soil dwellers, symbiotic relationships with protozoan and prokaryotic fauna within their gut help them turn over the complex biopolymer of wood and other cellulosic and lignocellulosic foodstuffs [73,75]. Termites are more potent in cellulose degradation and assimilation than other cellulose utilizing invertebrates [69]. Termites are also found to utilize fungus derived cellulolytic enzyme by making an intriguing symbiotic relationship with fungal species [76]. Cellulolytic gut bacteria have been screened in many species of termites, including *Zootermopsis angusticollis* [75], *Nasutitermes lujae* [77], *Macrotermes gilvus* [78], *Coptotermes gestroi* [79], *Cryptotermes sp.* [80], *Coptotermes formosanus* [81], *Coptotermes heimi* [82], *Cryptotermes brevis* [83], *Psammotermes hypostoma* Desneux [84], *Amitermes evuncifer* [85], *Macrotermes gilvus* [86], *Coptotermes curvignathus* [87].

The gut of Scarabaeidae beetle larvae is considered a potent bioreactor for the conversion of lignocellulosic materials to biofuels [88]. Scarabaeids larvae are humivorous feeding on soil organic matter, decaying plant roots, and woods, which are digested by the enzyme-producing microorganisms inhabiting within their digestive tract. The cellulolytic bacterial community has been screened within the larval gut of several Scarabaeidae beetle larvae, including *Pachnoda marginata* [89], *Holotrichia parallela* [30,90], *Oryctes rhinoceros* [91-93], *Lepidiota mansueta* [94], *Euoniticellus intermedius* [95], *Anamola dimidiata* [96]. Apart from Scarabaeids, other insect larvae such as *Dendroctonus armandi* (Curculionidae) [97], *Osphranteria coerulescens* (Cerambycidae) [98], banana pseudostem weevil *Odoiporus longicollis* (Coleoptera) [99] are also the host of the cellulose-degrading gut microbiome. Cellulolytic bacteria of five genera have been isolated from the larval gut of the moth *Diatraea saccharalis* [100,101]. The larvae of silkworm *Bombyx mori* feed on mulberry leaves composed of pectin, xylan, cellulose, and starch. And thus, *Bombyx mori* larvae also depend on gut bacteria for their dietary cellulose degradation [102,103]. Honey bees (*Apis mellifera*) are also considered as model organisms for the study of saccharide digestive gut microbiota [104]. Worker honey bees produce honey and bee bread by processing nectar and pollen, respectively. The honey and bee bread production mechanism depends on saccharide digestive enzymes produced by the gut microbiome of honey bees [104]. Other insects like silver crickets *Lepisma sp.* [105], mole crickets *Gryllotalpa africana* [106], rice weevil *Sitophilus oryzae* [107], coffee

berry borer *Hypothenemus hampei* [108], desert locust *Schistocerca gregaria* [109] also host gut microbes that degrade cellulosic foodstuffs.

Most of the termite species utilize microbial cellulase for degradation of the cellulosic foodstuffs, but the existence of endogenous cellulase has been reported within the gut of subterranean termite *Reticulitermes speratus* [110]. Apart from termite, endogenous cellulase activity has been found in other insect order also [19]. Most of the study has prioritized isolation and quantification of cellulolytic bacteria from different insect gut regions; some work has been focused on metagenomic and pyrosequencing approaches to identify cellulase-encoding genes. Termites are the insects in which cellulase genes have been first discovered [111], followed by other insect species belonging to the order Coleoptera [112,113], Hymenoptera [114], Orthoptera [115], and Hemiptera. β -glucosidase and endo- β -1,4 glucanase activities have been estimated in the gut of *Nasutitermes takasagoensis* [116]. Moreover, through the metagenomic approach, 45 different glycoside hydrolases (GH family) genes have been reported in higher termite *Nasutitermes takasagoensis* [117]. Researchers have identified endogenous cellulolytic systems within the beetle larvae also. With the aid of transcriptomic technology, one cellulase of glycoside hydrolase family 45 (GH45) and seven GH5 cellulases have been identified from the beetle larvae of *Mesosa myops* [118]. Two β -glycosidases (β Gly1 and β Gly2) have been purified from the midgut lumen of beetle *Tenebrio molitor* larvae [119]. Endogenous cellulase activities have also been detected in the gut homogenate of several cockroach species [64,120]. Cellulose digesting activity has also been determined in the digestive fluids of some other insects, including grasshopper *Dissosteira carolina* [121] and *Schistocerca gregaria* [64], longhorn beetle *Hylotrupes bajules*, Crickets *Acheata domesticus*, Stick insects *Eurycanta calcarata* [64], and locusts species [122].

3.2. Annelids.

Soil and plant litter dwelling earthworms are also known to possess glucose degrading enzymatic machinery within their gut. Microbial assemblages within the earthworm gut and casts facilitate enzymatic processing and mineralization of organic polymer of soil and plant biomass [123]. Cellulose degrading microbial community has been isolated from several species of earthworm, which include *Eudrilus eugeniae* [124-126], *Amyntas heteropoda* [127], *Eisenia fetida* [127-129], *Perionyx excavatus*, and *Glyphidrilus spelaeotes* [130]. Earthworms also rely on dual digestive mechanisms involving both endogenous and microbial cellulase for lignocellulose degradation. Few reports demonstrate that earthworms possess complete enzymatic machinery for glycosidic enzymes [131-134]. Glycolytic activities in the gut have been detected in the earthworm species *Pontoscolex corethrums*[118], *Millsonia anomala* [132], *Polypheretima elongata* [133], *Hormogaster elisae* [134, 135], *Hyperiodrilus africaizus*, *Dichogaster terrae nigrae* [135], *Pheretima hilgendorfi* [136]. N-acetylglucosaminase, laminarinase, laminaribiase activities are found to be most potent within the gut of these earthworm species except *Pheretima hilgendorfi*. These enzymes induce degradation of β -1, 3 glucan, and chitin sub-units, which are characteristic components of fungal cell walls [131,134]. Higher activities of these enzymes corroborate that these earthworm species feed on fungus and decaying root exudates. Weak activities of other glycolytic enzymes within the gut of earthworms reflect their dependency on microbial cellulases for degradation of substrates like mannan and cellulose [134]. In the case of *Pheretima hilgendorfi*, endo- β -1, 4-glucanase contributes to the degradation of cellulose, and a novel cellulase gene (phhEg) has been detected from this species [136].

3.3. Molluscs.

Apart from insects, some empirical studies have also been conducted to determine cellulolytic bacteria in snail species. Land snails (Gastropoda: Pulmonata) include several distinct lineages of terrestrial gastropods, which utilize various resources of the terrestrial ecosystem that make them efficient in exploiting the available niches, which is why the realized diversity is quite high. They are generally herbivorous, feed upon a wide range of plant materials, and many of them are the pests of agricultural and horticultural plants [137]. As most land snail species consume cellulosic and lignocellulosic materials, they can be a viable and potential source of cellulolytic gut microbe fauna. Pioneered study on bacterial cellulase in the animal gut has been conducted on land snail *Helix pomatia* [138,139], which has been followed by Florin and Lozet 1949 [140] and Jeuniaux 1950, 1955 [141,142], who worked on the contribution of microbial cellulase and chitinase respectively in the degradation of plant material in the gut of *H. pomatia*. The African giant snail *Achatina fulica* (Mollusca–Gastropoda) is the most studied snail species in this respect. The existence of endogenous cellulase within the gut of *A. fulica* is evident from the work of Soedigdo et al. 1970 [143] and Dar et al. 2020 [144]. Microbial communities with cellulolytic potential have been isolated from *Achatina fulica* [7,145-147] and *Arachatina marginata* [148,149]. Few works have been aimed to investigate the physiochemical environment of the gut of helioid snails [150], the occurrence of fermentative bacteria in edible snail *Helix pomatia* and *Cornu aspersum* (Gastropoda: Pulmonata) [151], and homolactic intestinal bacteria of *Helix aspersa* [152], but the detailed works emphasizing microbial contribution in the digestion of cellulose biopolymer in several other gastropod snail guts are yet to be deciphered. Other molluscan species such as marine turban shell *Batillus cornutus* has been found to possess polysaccharide digesting gut bacteria [153] and wood-boring bivalvia *Bankia setacea* also depends on nitrogen-fixing cellulolytic endosymbionts for wood degradation in the marine environment [154-157]. The cellulolytic activity within the different areas of the gut of the land slug *Arion ater* had been detected through the CMC zymography and esculin hydrate activity gel assays, which revealed the existence of endoglucanase and β -glucosidase enzymes [158] within their gut. Further study was carried out to isolate and identify cellulolytic bacterial colony within the *Arion* gut, which was the main source of enzyme activity within the gut [158]. Four endo- β -1,4-glucanases (21 K, 45 K, 65 K, and 95 K cellulase) and 2 β -glucosidases (110 K and 210 K) were purified from the digestive fluid of sea hare *Aplysia kurodai* [159]. These enzymes were able to hydrolyze CMC, filter papers, and lichenan, and these all cellulase were able to digest seaweeds, mainly sea lettuce *Undaria pinnatifida* [159].

Literature survey reflects that insect species are prioritized for the investigation of the gut bacterial community. Other phytophagous species such as terrestrial snails or algae or seaweed consuming aquatic snails and geophagous earthworms can also be efficient model species. Exploration of more invertebrate species may be helpful for the discovery of novel microorganisms with cellulolytic potentials. Lists of lower invertebrates and their gut bacterial strains with cellulolytic potentials (Table 1) and the specific activity of gut bacterial cellulolytic enzymes (Table 2) are presented in this review.

4. Biotechnology and Industrial Application of Cellulase

Biotechnological approaches have been adopted in the long past since the 1980s to apply cellulase in the food industry, followed by several other commercial and industrial parts

[1]. Commercially available cellulolytic enzymes are usually extracted from *Trichoderma reesei* and *Aspergillus niger* [1].

Table 1. List of several lower invertebrate species that host gut microbes with cellulolytic potential.

Species name	Systematic position	Bacterial strains identified	Cellulolytic enzyme activity of the culture Supernatant of the isolated bacterial strains	Reference
Termite (<i>Odontotermes hiananensis</i>), pill-bugs (<i>Armadillidium sp.</i>), yellow stem borers (<i>Scirpophaga incertulas</i>)	Arthropoda-Insecta-Blattodea-Termitidae: <i>Odontotermes hiananensis</i> , Arthropoda-Crustacea-Malacostraca-Isopoda: <i>Armadillidium sp.</i> , Arthropoda-Insecta-Lepidoptera-Crambidae: <i>Scirpophaga incertulas</i>	Bacterial families isolated belong to Bacillaceae, Enterobacteriaceae, Microbacteriaceae, Paenibacillaceae and Promicromonosporaceae	endoglucanase, exoglucanase, β -glucosidase, xylanase, b-xylosidase, mannanase and b-D-glucanase.	[2]
Termite (<i>Zootermopsis angusticollis</i>)	Arthropoda-Insecta-Blattodea-Termopsidae	Among several isolates, <i>Cellulomonas sp.</i> , <i>Bacillus</i> (e.g. <i>B. cereus</i> and <i>B. megaterium</i>), and <i>Paenibacillus sp.</i> were with highest CMC degrading capability	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in plate assay method.	[75]
Wood-feeding Termite, <i>Nasutitermes lujae</i>	Arthropoda-Insecta-Blattodea-Termitidae	<i>Clostridium termitidis sp.</i>	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[77]
Termite worker <i>Macrotermes gilvus</i>	Arthropoda-Insecta-Blattodea-Termitidae	<i>Bacillus megaterium</i> and <i>Paracoccus yeei</i>	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[78]
Milk termite (<i>Coptotermes gestroi</i>)	Arthropoda-Insecta-Blattodea-Rhinotermitidae	<i>Bacillus sp.</i> , <i>Enterobacter sp.</i> , <i>Bacillus megaterium</i> , <i>Pseudomonas aeruginosa</i> and <i>Bacillus cereus</i>	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[79]
<i>Cryptotermes sp.</i>	Arthropoda-Insecta-Blattodea-Rhinotermitidae	Three isolates of genus <i>Clostridium</i> , one isolate of group Mycobacteriaceae, Lactobacillaceae or Coryneform, and the last one in the genus <i>Proteus</i>	enzyme assay was not performed, Cellulolytic enzyme activities had been screened based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[80]
Termite <i>Coptotermes formosanus</i>	Arthropoda-Insecta-Blattodea-Rhinotermitidae	<i>Pseudomonas mendocina</i> , <i>Burkholderia pseudomallei</i> , <i>Chryseobacterium luteola</i> , <i>Klebsiella oxytoca</i> and <i>Klebsiella terrigena</i>	filter paperase (The cellulolytic enzyme activity of the microbe was examined in a broth culture using filter paper as carbon source)	[81]
Termite <i>Coptotermes heimi</i>	Arthropoda-Insecta-Blattodea-Rhinotermitidae	<i>Bacillus sp.</i> , <i>Proteus sp.</i> , <i>Ochrobactrum sp.</i> , <i>Erwinia sp.</i> , <i>Aeromonas sp.</i> and <i>Citrobacter sp.</i>	enzyme assay was not performed, Cellulolytic enzyme activities had been screened based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[82]
Termite <i>Cryptotermes brevis</i>	Arthropoda-Insecta-Blattodea-Kalotermitidae	<i>Bacillus sp.</i> and <i>Ochrobactrum oryzae</i>	xylanase, CMCase, lignin peroxidase, laccase	[83]

Species name	Systematic position	Bacterial strains identified	Cellulolytic enzyme activity of the culture Supernatant of the isolated bacterial strains	Reference
Termite <i>Psammotermes hypostoma</i> Desneux	Arthropoda-Insecta-Blattodea-Rhinotermitidae	<i>Paenibacillus lactis</i> , <i>Lysinibacillus macrolides</i> , <i>Stenotrophomonas maltophilia</i> , <i>Lysinibacillus fusiformis</i> and <i>Bacillus cereus</i>	cellulase (endoglucanase)	[84]
Termite <i>Amitermes evuncifer</i>	Arthropoda-Insecta-Blattodea-Termitidae	<i>Bacillus cereus</i> , <i>Bacillus mycooides</i> and <i>Pseudomonas aeruginosa</i>	endoglucanase (CMCase) and exoglucanase (FPase) and	[85]
Termite <i>Macrotermes gilvus</i>	Arthropoda-Insecta-Blattodea-Termitidae	<i>Providencia sp.</i> , <i>Bacillus sp.</i>	cellulase (on newsprint paper substrate)	[86]
Termite <i>Coptotermes curvignathus</i>	Arthropoda-Insecta-Blattodea-Termitidae	Bacterial strains isolated were mainly <i>Bacillus spp.</i>	enzyme assay was not performed, Cellulolytic enzyme activities had been screened based on the clear zone diameter of degraded CMC area around the colony in the plate assay method	[87]
Termite	Arthropoda-Insecta	<i>Dipolcocci sp.</i> , <i>Diplobacilli sp.</i> , <i>Streptobacilli sp.</i> and <i>Staphylococci sp.</i>	enzyme assay was not performed, Cellulolytic enzyme activities had been screened based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[160]
<i>Holotrichia parallela</i> larvae	Arthropoda-Insecta-Coleoptera-Scarabaeidae	Among many isolates <i>Siphonobacter aqua</i> <i>eclarae</i> , <i>Cellulosi microbium</i> <i>funkei</i> , <i>Paracoccus</i> <i>sulfuroxidans</i> , <i>Ochrobactrum</i> <i>cytisi</i> , <i>Ochrobactrum</i> <i>haematophilum</i> , <i>Kaistia adipata</i> , <i>Devosia riboflavina</i> , <i>Labrys neptuniae</i> , <i>Ensifer adhaerens</i> , <i>Shinella zoogloeoides</i> , <i>Citrobacter freundii</i> and <i>Pseudomonas nitroreducens</i> were reported for the first time as cellulolytic bacteria	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[30]
Long horn beetle <i>Hylotrupes bajules</i>	Arthropoda-Insecta-Coleoptera-Cerambycidae	Not identified	β -glycosidase, CMC-ase, xylanase	[64]
Larvae of the scarab beetle <i>Pachnoda marginata</i>	Arthropoda-Insecta-Coleoptera-Scarabaeidae	<i>Promicromonospora pachnodae</i> <i>sp.</i>	CMC-ase and xylanase	[89]
<i>Holotrichia parallela</i> larvae	Arthropoda-Insecta-Coleoptera-Scarabaeidae	<i>Pseudomonas sp.</i>	endoglucanase	[90]
Larvae of <i>Oryctes rhinoceros</i>	Arthropoda-Insecta-Coleoptera-Scarabaeidae	Genus <i>Bacillus</i> and <i>Citrobacter</i>	enzyme assay was not performed. Cellulolytic, Xylanolytic, and Mannanolytic enzyme activities had been screened based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[91]
Larvae of <i>Oryctes rhinoceros</i>	Arthropoda-Insecta-Coleoptera-Scarabaeidae	<i>Bacillus sp.</i> , <i>Proteus sp.</i> , <i>Ochrobactrum sp.</i> , <i>Erwinia sp.</i> , <i>Aeromonas sp.</i> , <i>Citrobacter sp.</i> and <i>Pseudomonas sp.</i>	enzyme assay was not performed, Cellulolytic and ligninolytic enzyme activities had been screened based on the clear zone diameter area around the colony in the plate assay method.	[92]
Larvae of grub beetle <i>Lepidiota mansueta</i>	Arthropoda-Insecta-Coleoptera-Scarabaeidae	<i>Citrobacter sp.</i>	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear	[94]

Species name	Systematic position	Bacterial strains identified	Cellulolytic enzyme activity of the culture Supernatant of the isolated bacterial strains	Reference
			zone diameter of degraded CMC area around the colony in the plate assay method.	
Dung beetle <i>Euoniticellus intermedius</i>	Arthropoda-Insecta-Coleoptera-Scarabaeidae	Not identified	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[95]
Larvae of <i>Anamola dimidiata</i>	Arthropoda-Insecta-Coleoptera-Scarabaeidae	The majority of the isolated strain belonged to Firmicutes and Proteobacteria	endoglucanase, exoglucanase, β -glucosidase	[96]
Larvae of <i>Dendroctonus armandi</i>	Arthropoda-Insecta-Coleoptera-Curculionidae-Scolytinae	<i>Serratia sp.</i> , <i>Pseudomonas sp.</i> , <i>Bacillus sp.</i> , <i>Paenibacillus sp.</i> , <i>Sphingomonas</i> , <i>Brevundimonas sp.</i> , <i>kwangchunensis sp.</i> , <i>Brevundimonas vesicularis</i> , <i>Pseudoxanthomonas mexicana</i> and <i>Methylobacterium populi</i>	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[97]
Larvae of <i>Osphranteria coerulea</i>	Arthropoda-Insecta-Coleoptera-Cerambycidae	<i>Bacillus sp.</i>	CMC-ase	[98]
Banana pseudostem weevil <i>Odoiporus longicollis</i>	Arthropoda-Insecta-Coleoptera-Curculionidae	Not identified	CMCase	[99]
Larvae of moth <i>Diatraea saccharalis</i>	Arthropoda- Insecta-Lepidoptera-Crambidae	<i>Klebsiella oxytoca</i> , <i>Klebsiella pneumoniae</i> , <i>Klebsiella variicola</i> , <i>Stenotrophomonas maltophilia</i> , <i>Stenotrophomonas rhizophila</i> , <i>Bacillus pumilus</i> , <i>Enterococcus casseliflavus</i> , <i>Microbacterium hominis</i> and <i>Microbacterium schleiferi</i> ,	CMC-ase	[100]
Larvae of moth <i>Diatraea saccharalis</i>	Arthropoda-Insecta-Lepidoptera-Crambidae	<i>Klebsiella pneumoniae</i> , <i>Klebsiella sp.</i> and <i>Bacillus sp.</i>	CMC-ase	[101]
Larvae of <i>Bombyx mori</i>	Arthropoda-Insecta-Lepidoptera-Bombycidae	<i>Bacillus circulans</i> , <i>Proteus vulgaris</i> , <i>Klebsiella pneumoniae</i> , <i>Enterobacter sp.</i> , <i>Citrobacter freundii</i> and <i>Serratia liquefaciens</i>	cellulase, xylanase, amylase, pectinase	[102]
<i>Bombyx mori</i>	Arthropoda-Insecta-Lepidoptera-Bombycidae	<i>Solibacillus silvestris</i> , <i>Bacillus aryabhatai</i> , <i>Lysinibacillus sp.</i> , <i>Bacillus sp.</i> , <i>Bacillus thuringiensis</i> , <i>Paenibacillus sp.</i> , <i>Serratia marcescens</i> , <i>Klebsiella pneumoniae</i> and <i>Enterobacter hormaechei</i>	CMC-ase	[103]
Silver cricket <i>Lepisma sp.</i>	Arthropoda-Insecta-Zygentoma-Lepismatidae	Not identified	filter paperase (The cellulolytic enzyme activity of the microbe was examined in a broth culture using Whatman 42 filter as carbon source)	[105]
Mole crickets <i>Gryllotalpa africana</i>	Arthropoda-Insecta-Orthoptera-Gryllotalpidae	<i>Acinetobacter junii</i>	CMC-ase	[106]
Rice weevil <i>Sitophilus oryzae</i>	Arthropoda-Insecta-Coleoptera-Curculionidae	Bacterial strains isolated belongs to <i>Bacillus</i> and γ - <i>Protobacteria</i>	endoglucanase (CMCase)	[107]

Species name	Systematic position	Bacterial strains identified	Cellulolytic enzyme activity of the culture Supernatant of the isolated bacterial strains	Reference
Coffee berry borer <i>Hypothenemus hampei</i>	Arthropoda-Insecta-Coleoptera-Curculionidae	Based on morphological and biochemical characteristics, isolated strain was similar to genus <i>Brochothrix</i>	cellulase (CMCase)	[108]
Desert locust <i>Schistocerca gregaria</i>	Arthropoda-Insecta-Orthoptera-Acrididae	<i>Bacillus safensis</i>	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[109]
Termite, caterpillar, bookworm and snail	Arthropoda-Insecta (termite, caterpillar, bookworm) and Mollusca (snail)	Not identified	filter paperase and endoglucanase	[161]
<i>Oxya velox</i> , <i>Aspidimorpha miliaris</i> and <i>Propylea quatuordecimpunctata</i>	Arthropoda-Insecta-Orthoptera-Acrididae: <i>Oxya velox</i> , Arthropoda-Insecta-Coleoptera-Chrysomelidae: <i>Aspidimorpha miliaris</i> , Arthropoda-Insecta-Coleoptera-Coccinellidae: <i>Propylea quatuordecimpunctata</i>	Bacterial species isolated from <i>O. velox</i> were <i>Photorhabdus luminescens</i> , <i>Enterococcus faecalis</i> , <i>Enterococcus durans</i> , <i>Flavobacterium odoratum</i> , <i>Serretia marcescens</i> and <i>Serretia entomophila</i> . Isolates identified from <i>P. quatuordecimpunctata</i> were <i>Erwinia ananus</i> , <i>Aeromonas salmonicida</i> , <i>Enterococcus casseliflavus</i> and <i>Acinetobacter calcoaceticus</i> . Isolates identified from <i>A. miliaris</i> were <i>Klebsiella oxytoca</i> , <i>Microbacterium imperiale</i> , <i>Yersinia pestis</i> , <i>Xenorhabdus poinari</i> and <i>Pseudomonas saccharophila</i>	enzyme assay was not performed, Cellulolytic bacterial strains were isolated based on the clear zone diameter of degraded CMC area around the colony in the plate assay method.	[162]
Endogeic earthworms, <i>Amyntas heteropoda</i> and <i>Eisenia fetida</i>	Annelida-Clitellata-Haplotaxida-Megascolecidae: <i>Amyntas heteropoda</i> , Annelida-Clitellata-Haplotaxida-Lumbricidae: <i>Eisenia fetida</i>	Dominant bacterial and fungal genus was <i>Burkholderia</i> and <i>Chaetomium</i> respectively	exoglucanase, endoglucanase, xylanase, laccase	[123]
Earthworms <i>Eudrilus eugeniae</i>	Annelida-Clitellata-Haplotaxida-Eudrilidae	<i>Bacillus pumilus</i>	endoglucanase	[124]
Earthworm <i>Eudrilus eugeniae</i>	Annelida-Clitellata-Haplotaxida-Eudrilidae	<i>Bacillus</i> sp.	amylase, nitrate reductase, cellulase, xylanase, and protease	[125]
Earthworm <i>Eisenia foetida</i>	Annelida-Clitellata-Haplotaxida-Lumbricidae	<i>Lysinibacillus sphaericus</i>	filter paperase	[128]
Earthworm <i>Eisenia fetida</i>	Annelida-Clitellata-Haplotaxida-Lumbricidae	Colony of <i>Streptococcus</i> , <i>Staphylococcus</i> and <i>Diplococcus</i>	CMC -ase	[129]
Epigeic earthworm, <i>Perionyx excavatus</i> and an endogeic, <i>Glyphidrilus spelaeotes</i>	Annelida-Clitellata-Haplotaxida-Megascolecidae: <i>Perionyx excavatus</i> , Annelida-Clitellata-Haplotaxida-Almidae: <i>Glyphidrilus spelaeotes</i>	<i>Mycobacterium</i> sp., <i>Stenotrophomonas</i> sp., <i>Acinetobacter</i> sp., <i>Alcaligenes</i> sp., <i>Chryseobacterium</i> sp., <i>Acinetobacter</i> sp., <i>Pseudomonas</i> sp., <i>Bacillus</i> sp. and <i>Sphingomonas</i> sp.	Filter paperase	[130]
Giant African land snail <i>Achatina fulica</i>	Mollusca- Gastropoda-Stylommatophora-Achatinidae	<i>Bacillus subtilis</i> , <i>Achromobacter</i> , sp., <i>Ochrobactrum</i> sp. and <i>Klebsiella</i> sp.	endoglucanase, exoglucanase, xylanase	[14]

Species name	Systematic position	Bacterial strains identified	Cellulolytic enzyme activity of the culture Supernatant of the isolated bacterial strains	Reference
Giant African land snail <i>Achatina fulica</i>	Mollusca- Gastropoda- Stylommatophora- Achatinidae	Many genera had been isolated which were belonged to three phyla, namely <i>Proteobacteria</i> , <i>Actinoibacteria</i> , and <i>Firmicutes</i>	p-nitrophenyl-b-D-cellobioside(pNPC), 4-methylumbelliferyl-b-D-cellobioside(MUC), 4-methylumbelliferyl-b-D-glucopyranoside(MUG), p-nitrophenyl-b-D-glucopyranoside(pNPG), 4-methylumbelliferyl-b-D-xylopyranoside(MUX), powdered sugarcane bagasse and CMC hydrolyzing enzymes (Enzyme activities were detected by plate assay mehod)	[145]
Giant African land snail <i>Achatina fulica</i>	Mollusca- Gastropoda- Stylommatophora- Achatinidae	Not identified	CMC-ase	[146]
Giant African land snail <i>Achatina fulica</i>	Mollusca- Gastropoda- Stylommatophora- Achatinidae	<i>Micrococcus sp.</i> , <i>Enterobacter sp.</i> and <i>Yokenella sp.</i>	CMC-ase, filter paperase, Xylanase	[147]
Giant African snail <i>Archachatina marginata</i>	Mollusca- Gastropoda- Stylommatophora- Achatinidae	<i>Bacillus subtilis</i> , <i>Streptococcus casseliflavus</i> , <i>Streptococcus faecalis</i> and <i>Staphylococcus aureus</i> ,	CMC-ase, protease	[148]
Giant African land snail <i>Archachatina marginata</i>	Mollusca- Gastropoda- Stylommatophora- Achatinidae	<i>Staphylococcus aureus</i> , <i>Baccillus subtilis</i> , <i>Streptobacillus sp.</i> , <i>Streptococcus aureus</i> and <i>Escherichia coli</i> ,	cellulase, α -glucosidase, amylase, proteinase, and lipase	[149]
Marine turban shell <i>Batillus cornutus</i>	Mollusca- Gastropoda- Trochida-Turbinidae	<i>Bacillus sp.</i> and <i>Staphylococcus sp.</i>	carboxymethyl cellulase, α -cellulase, laminarinase and kelp-lyase	[153]

Table 2. Specific activity of several cellulolytic enzymes obtained from gut microbial flora of several lower invertebrate species.

Invertebrate species	Gut microbial flora	Specific activity of enzyme obtained from gut microbial flora (maximum activities showed within the incubation period of bacteria culture, are mentioned here)	Reference
Termite <i>Cryptotermes brevis</i>	<i>Bacillus sp.</i>	xylanase activity: 0.21 U/mL CMCase activity: 0.25 U/mL	[83]
	<i>Ochrobactrum oryzae</i>	lignin peroxidase activity: 14.6 IU/mL laccase activity: of 8 IU/mL	
Termite <i>Psamotermes hypostoma</i>	<i>Paenibacillus lactis</i>	endoglucanase activity: 1.47 U/ml	[84]
	<i>Lysinibacillus fusiformis</i>	endoglucanase activity: 0.22 U/ml	
	<i>Stenotrophomonas maltophilia</i>	endoglucanase activity: 2.28 U/ml	
	<i>Lysinibacillus macrolides</i>	endoglucanase activity: 1.93 U/ml	
	<i>Bacillus cereus</i>	endoglucanase activity: 0.23 U/ml	
Termite <i>Amitermes evuncifer</i>	<i>Bacillus cereus</i>	endoglucanase activity: 6.38 $\mu\text{mol min}^{-1}\text{mg}^{-1}$ exoglucanase activity: 1.14 $\mu\text{mol min}^{-1}\text{mg}^{-1}$	[85]
	<i>Bacillus mycoides</i>	endoglucanase activity: 5.96 $\mu\text{mol min}^{-1}\text{mg}^{-1}$ exoglucanase activity: 1.08 $\mu\text{mol min}^{-1}\text{mg}^{-1}$	
	<i>Pseudomonas aeruginosa</i>	endoglucanase activity: 4.89 $\mu\text{mol min}^{-1}\text{mg}^{-1}$ exoglucanase activity: 1.47 $\mu\text{mol min}^{-1}\text{mg}^{-1}$	
Termite <i>Macrotermes gilvus</i>	<i>Providencia sp.</i>	cellulase activity: 15.7 mU/mL	[86]
	<i>Bacillus sp.</i>	cellulase activity: 2.33 mU/mL	
Termite <i>Nasutitermes takasagoensis</i>	Not identified	endoglucanase (CMCase) activity: 2.40 units/mg β -glucosidase (cellobiase) activity: 0.36 units/ mg (one unit is the amount of enzyme that produce 1 μmol glucose or glucose equivalent/min)	[116]
Termite, caterpillar and book worm	Not identified	endoglucanase activity: 0.400 IU/mL extract filter papersae activity: 0.194 IU/mL extract	[160]
Termite	Not identified	CMC-ase activity: 0.0155 IU/ml filter paperase activity: 0.004 IU/ml	[163]
<i>Holotrichia parallela</i> larvae	<i>Pseudomonas sp.</i>	endoglucanase activity: 0.825 U/mL	[90]

Beetle <i>Osphranteria coerulescens</i> larvae	<i>Bacillus sp.</i>	CMC-ase activity: 4.99 U/mL	[98]
Moth <i>Diatraea saccharalis</i> larvae	<i>Bacillus pumilus</i>	CMC-ase activity: 0.32 U/mL enzyme activity on sugarcane biomass: 0.23 U/mL	[100]
	<i>Klebsiella oxytoca</i>	CMC-ase activity: 0.22 U/mL enzyme activity on sugarcane biomass: 0.13 U/mL	
Moth <i>Diatraea saccharalis</i> larvae	<i>Klebsiella pneumoniae</i>	cellulase activity of protein extract: 30.13 U/mg	[101]
	<i>Klebsiella sp.</i>	cellulase activity of protein extract: 19.97 U/mg	
	<i>Bacillus sp.</i>	cellulase activity of protein extract: 5.53 U/mg	
Sikworm <i>Bombyx mori</i> larvae	<i>Bacillus aryabhatai</i>	cellulase activity: 0.4 U/mL (values is approximated from the graphical representation)	[103]
Mole crickets <i>Gryllotalpa africana</i>	<i>Acinetobacter junii</i>	CMCase activity: 0.35 U/ml	[106]
Rice weevil <i>Sitophilus oryzae</i>	<i>Bacillus subtilis</i>	cellulase (endoglucanase activity) 132.069 ± 0.993 U/mL	[107]
Earthworm <i>Eudrilus eugeniae</i>	<i>Bacillus pumilus</i>	cellulase (endoglucanase) activity: 0.1271 IU/mL	[124]
Earthworm <i>Eisenia foetida</i>	<i>Lysinibacillus sphaericus</i>	cellulase activity 1.92 FPU/mL (cellulase activity was expressed here in terms of Filter Paper Units (FPU))	[128]
	Not identified	CMC-ase activity: 26.041 IU/mL and 47.80 IU/mL produced by two different culture	[129]
Epigeic earthworm, <i>Perionyx excavatus</i> and an endogeic, <i>Glyphidrilus spelaeotes</i>	<i>Mycobacterium sp.</i> <i>Stenotrophomonas sp.</i> <i>Acinetobacter sp.</i> <i>Alcaligenes sp.</i> <i>Chryseobacterium sp.</i> <i>Acinetobacter sp.</i> <i>Pseudomonas sp.</i> <i>Bacillus sp.</i> <i>Sphingomonas sp.</i>	The highest cellulase (filter paperase) activity of these carbohydrate degrading bacteria was ranged from 0.42 to 0.59 μM glucose ml ⁻¹ min ⁻¹ (The cellulase enzyme activity was determined here as glucose equivalent)	[130]
<i>Achatina fulica</i>	<i>Bacillus subtilis</i>	endoglucanase activity: 230.86 IU/mL gut extract for CMC substrate	[14]
	<i>Ochrobactrum sp.</i>	endoglucanase activity 502.75 IU/mL gut extract for grass straw as substrate 347.65 IU/mL gut extract for wheat husk as a substrate 112.68 IU/mL gut extract for filter paper as a substrate	
	<i>Bacillus subtilis</i>	exoglucanase activity: 3777.61 IU/mL extract for filter paper as a substrate	
	<i>Ochrobactrum sp.</i>	exoglucanase activity: 2406.31 IU/mL extract for wheat husk as a substrate	
	<i>Bacillus subtilis</i>	xylanase activity: 60.22 IU/mL extract) on wheat husk as a substrate	
	<i>Ochrobactrum sp.</i>	xylanase activity: 82.03 IU/mL extract for grass straw as substrate 24.23 IU/ mL extract for filter paper as a substrate	
	<i>Aspergillus niger</i>	cellulase (CMCase) activity from fungal isolates 14.46 mg/ml sec ⁻⁴	
<i>Achatina fulica</i>	Not identified	CMCase activity: 0.4539 U/mL	[146]
<i>Achatina fulica</i>	<i>Enterobacter sp.</i>	filter paperase activity: 5 U/ml CMC-ase activity: 2.5 U/ml xylanase activity: 0.6 U/ml (values are approximated from the graphical representation)	[147]
	<i>Yokenella sp.</i>	filter paperase activity: 3 U/ml CMC-ase activity: 4 U/ml xylanase activity: 0.7 U/ml (values are approximated from the graphical representation)	
<i>Archachatina marginata</i>	<i>Bacillus subtilis</i>	cellulase (CMCase) activity: 2.2 mg/mL sec ⁻⁴	[148]
	<i>Streptococcus casseliflavus</i>	cellulase (CMCase) activity: 1.7 mg/mL sec ⁻⁴	
	<i>Streptococcus faecalis</i>	cellulase (CMCase) activity: 1.4 mg/mL sec ⁻⁴	
	<i>Staphylococcus aureus</i>	cellulase (CMCase) activity: 0.2 mg/mL sec ⁻⁴	
<i>Achatina marginata</i>	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Streptobacillus sp.</i> , <i>Streptococcus aureus</i> , <i>Escherichia coli</i>	amylase activity: 18.40 mg/g lipase activity: 15.80 mg/g cellulase activity: 13.20 mg/g proteinase activity: 13 mg/g α-glucosidase activity: 8.30 mg/g	[149]

		(enzyme activity was assessed from the gut homogenate, maximum enzyme activity of adult snails are mentioned here)	
Sea snail <i>Batillus cornutus</i>	<i>Bacillus sp.</i>	CM-cellulase activity: 22.76 U/mg protein α -cellulase activity: 27.10 U/mg protein laminarinase activity: 66.59 U/mg protein kelp-lyase activity: 64.36 U/mg protein	[153]

To obtain efficient hydrolytic potential, cellulase enzymes should possess some desired attributes, including high specific activity, high catalytic activity against crystalline cellulose, high thermostability, resistance to end-product inhibition, and stability against shear force [164]. Various genetic tools are being used for microbial strain improvement to achieve these attributes and enhance enzyme production. Several industrially used fungal strains such as *A. niger*, *T. reesei*, *Saccharomyces cerevisiae*, *Pichia pastoris*, and bacterial strains like *Escherichia coli*, *Bacillus subtilis* [164] have subjected to genetic engineering for the production of a recombinant enzyme with high potential for industrial application. Homologous and heterologous expression techniques have been adopted in the recent era to overexpress microbial cellulase and other hydrolytic enzymes [164]. Owing to the genetic engineering of the cellulolytic microbial strain, cellulose-degrading enzymes' efficient production has enhanced its biotechnological potential in various industrial fields. A brief account of the application of cellulase and allied enzymes have been discussed here.

4.1. Food processing industry.

The application of enzymes in the extraction of fruit juices and pulps mitigates the problem of low yield, stability, and clarity of product, which are the main difficulties faced by the food industries in the early 1930s. Later on, progressive research on enzyme technology leads to the production of cellulase, hemicellulase, and pectinase from the food-grade microorganisms *A. niger* and *T. reesei*. A combination of these enzymes (pectinase, cellulase, hemicellulase), also called macerating enzymes, plays an important role in the extraction and clarification of vegetable and fruit juices [1] also improves the stability and textures of the purees and pulp. A mixture of pectinase and a low level of hemicellulase and cellulase, commercially know as Olivex is used to extract olive oil from olive seeds. The use of Olivex improves the quality of olive oil extract by enriching extra virgin olive oil with vitamin E and antioxidants, reducing the induction of rancidity and lowering oil content in the wastewater [165]. Infusion of pectinase enzyme helps in peeling of citrus food by reducing its bitterness. Application of β -glucosidase and pectinase ameliorate the texture, aroma, flavor, and volatiles compounds of specific fruits and vegetables [166]. Microbial enzymes are long being used in the quality improvement of bakery products also. Amylases and proteases are mainly used in the bakery industry [167], but recently the use of hemicellulase and endo-xylanase helps in equal distribution of water in dough and bread by hydrolyzing arabinoxylan present in dough [168]. This redistribution of water facilitates the enhancement of flavor, volume, softness, texture, and bakery products' stability.

4.2. Brewery and winery industry.

The application of exogenous enzymes in wine and beer biotechnology plays a key role in quality control and production rate. α and β -amylase, carboxypeptidase, and β -glucanase are endogenously synthesized during the germination of barley before malting and synergistically act hydrolyze seed reserves during the malting process. But their improper activities often result in un-malted and poor quality barley. Application of microbial β -glucanase facilitates

hydrolysis of β -glucan and reduces the wort viscosity during the maceration and fermentation process of barley. In the winery, exogenous enzymes hemicellulase, pectinase, β -glucanase are used for better maceration, improved color extraction, filtration and clarification, and wine stability and quality [165]. Furthermore, the β -glucosidase enzyme application modifies glycosylated precursors that enhance the aroma of wine [169].

4.3. Paper and pulp industry.

Application of biomechanical pulping process using enzymes instead of the only mechanical process reduces the energy expenditure during grinding and refining of the woody material in pulps. Mixtures of endoglucanase I and II and hemicellulase have been used to better drainage and beat ability in the paper mills before or after beating pulp, which in turn increases the overall production rate [1]. Cellulase and xylanase enhance the bleaching and deinking of several types of paper wastes [170]. Overall addition of several hydrolytic enzymes ameliorates fiber brightness, strength properties, pulp freeness, and cleanliness.

4.4. Textile and laundry industry.

The application of cellulase in the bio-stoning process of denim and jeans products has achieved great success. Usage of cellulase in bio polishing of cotton fabric also has an advantage as an enzyme can readily remove surface fibers and fuzz, resulting in the glossy, smooth, and brighter appearance of cotton garments [1,165]. Cotton garments usually become fluffy and dull after repeated wash. The addition of cellulase enzyme in household detergents helps remove fluffy fibrils from cotton, boosting the appearance and brightness of the garments [1].

4.5. Animal feed.

In the animal feed industry, cellulase plays a key role in removing Anti-nutritional Factors (ANF) from the cereals, grains, and vegetables used for animal feed in poultry, cattle, and fish farming. Pretreatment with cellulase and hemicellulase induces partial digestion of lignocellulosic materials and β -glucans, dehulling cereal grains, which improves the cereal quality and ensures a high yield of milk and meat production [165].

4.6. Research development and agriculture.

A combination of hydrolytic enzymes, including cellulase, hemicellulase, ligninase, have an immense effect on plant growth and plant disease control [1]. Cellulases and β -glucanases can degrade the cell wall and inhibit the germination of spores of some phytopathogens. Mixtures of different hydrolytic enzymes facilitate the digestion of desired plant or fungal cell walls to produce protoplast, which can be used to make hybrid strains of desired properties for research purposes [23].

4.7. Waste management.

As cellulose is the most abundant biomolecules in the plant, a large number of wastes of leaf litter and other lignocellulosic materials are generated from forests, agricultural fields, and agro-industries. These wastes containing a large amount of raw cellulose may cause environmental pollution. But nowadays, with the help of enzyme technology, these unutilized

or underutilized cellulosic sources are being converted to produce several biofuels and bio commodities, sugars, and alcohol [1,171,172]. Application of garden snail (*Cornu aspersum*) cellulase in paper waste saccharification is empirical evidence of cellulase activity in waste management [173].

5. Conclusion and Future Prospect

Cellulase and allied enzymes are getting attraction worldwide due to their wide range of applications in vast areas of industries. Although in the past, fungal-based enzymatic systems have been used for cellulolytic enzyme production, later many research works have been carried out in search of more efficient microbial enzymatic systems as a source of cellulolytic enzymes. Bacterial enzymatic systems are more promising due to enzyme complexity, extreme habitat variability, and low production cost. Researchers are focusing on bacterial strain improvement to obtain tailor-made cellulolytic enzymes with high specific activity and catalytic efficiency with the aid of biotechnology and enzymology. Moreover, identifying newer sources of cellulose-degrading microorganisms is essential for the isolation of novel cellulolytic genes. Previous studies assert that the gut of phytophagous and herbivorous invertebrates is the host of the cellulolytic bacterial niche. In the future, further exploration of such invertebrates is necessary for the isolation of novel bacteria, which will bring great prospects in the industrial application of cellulolytic enzymes.

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Conflicts of Interest

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