Dung beetle gut microbes: Diversity, metabolic and immunity related roles in host system

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INTRODUCTION

Coleoptera is one of the largest order of insects with about 370,000 insect species described worldwide. Dung beetles are a major insect group (Coleoptera: Scarabaeinae) distributed globally except Antarctica with a high number of diversity comprising nearly 6,200 species and almost 267 genera [1]. These species are coprophagous in nature which live freely in soil and mostly feed on both wet and dry dung materials of herbivorous mammals. The undigested excreta of mammals are utilized as food and nesting material throughout their life cycle, hence, they possess many ecologically beneficial functions. It is believed that the quality and development of the larvae and adult depends on the quality and quantity of the brood prepared by beetles [2]. The dung beetles play a vital role in nutrient recycling by decaying organic matter and developing soil aeration [3,4] thereby, reducing the greenhouse gas fluxes [5,6]. It also enhances plant growth and grain production [7 - 9]. They increase the above ground biomass by means of burying dung [10]. Secondary seed dispersal [11,12] and suppressing gastrointestinal parasites etc. As an insect group, they possess an incredible innate immune system and hold multiple defense strategies such as possessing an epithelial barrier, synthesizing novel peptide like compounds against pathogenic microbes and releasing reactive oxygen species to protect them from the pathogenic environment [13].

The extensive role of dung beetles in various ecological functions and its innate immune system were much reviewed by many researchers [14 - 20]. Partially digested dung consists of more than 80 % indigestible materials like cellulose, lignin, tannin, chitin and other waste materials along with the pathogenic microbes from ruminant herbivore. Though the insect digestive system secretes a variety of digestive enzymes, many of them need to have a symbiotic microbiota because of the recalcitrant chemical composition of plant cell walls [21]. Hence, this review aims to give a breakthrough concept on the diversified role of gut microbes in scarab beetles in various aspects.

Habitats and diversity of gut microbes in Scarab beetles

Insects are the most widespread animal group having more diversity and density in their intestinal microbial population. Generally, polyphagous insects show more microbial diversity than the monophagous insects [22 - 26]. Scarab beetles mostly feed on dung, decaying wood or other plant materials, therefore, need strong intestinal materials to digest the tough diet. They own a typical gut design which provides several physicochemical properties like longer and alkaline midget having many stable alkaline hydrolases, specialized and broadened hindgut leading to the accumulation of millions of microbes. The basic layout of insect gut allows many alterations, reflecting variations according to specialized niches and feeding habits, with a lot of these specializations evolving for residence of intestine microorganisms in distinct gut compartments [27]. The surprising fact is that the insect gut contains 10 times more microbial cells than its host cell number and also have more than 100-fold microbial genes than the host gene [28,29].
Generally, hindgut region of insects is called as fermentation chamber or bioreactor because of their potential applications in digestive process. Scarab larval midget is remarkably alkaline in nature and as it is influenced by serine proteinase enzymes, which allows the dominance of the gut microbiota with cellulolytic potential [30, 31]. It was reported that frass producing insect gut was dominated by three domains of microbes- Archeaa, Eucarya and bacteria leading to the digestion of plant polymer [32]. The scarab beetle Pachyoda ephippia gut microbes holding a high number of Firmicutes, β Proteobacteria and Bacteroidetes shared an equal quantity followed by δ Proteobacteria and Bacillales [33]. Cultivable bacteria isolated from the Thoraceus luscianthus gut also revealed findings similar to Andert et al. (2010). [33] that the hindgut microbe population comprised 71% of Proteobacteria isolates which included γ, α, β Proteobacteria and 18% of Firmicutes and Actinobacteria [34].

Among the orders discussed above, Enterobacteriales consisting of Enterobacter, Escherichia, Shigella, Serratia, Citrobacter species [35] and Yersinia, Serratia and Rahnella are abundant in southern pine beetle hindgut [36] whereas the alkaline midget of humus feeding scarab larve P. ephippia showed the domination of Actinobacteria [37]. Recently one unique method of transmitting symbiotic microbes by the route of brood ball was identified in dung beetle Onthophagus Taurus [38]. Dung beetle gut is also acting as a successful residence of many beneficial yeasts in the way of offering extra nutrition, insect development, and detoxification of toxins [39, 40]. Eleven yeast species of Candida, Trichosporon, Galactomyces, and Schwannomyces were isolated from dung beetle Helicoptris buphelus Fabricius [41]. Recently, gut-associated fungal species along with bacterial species were identified in some wood-feeding scarab beetles [42]. These findings proved that the insects which are the inhabitant of varied food materials possess very diverse intestinal microbiome.

**Digestive role of gut microbes in Scarab beetle**

Digestion is an essential process in animals that grant the necessary nutrients getting absorbed from the waste materials which may govern vital activities including growth, development, reproduction and environmental adaptation of host insects [34, 43]. Dung is an incomplete source of nutrient because it lacks various essential amino acids like histidine, methionine, tryptophan, and arginine [44]. For the complete utilization of nutrients, the insects build a symbiotic relationship with commensal microbes and the complexity of microbes varies from species to species [45 - 47]. Yun et al, (2014). [48] Evidently disclosed that insect intestinal microbial diversity relied on diet, environmental habitat and different developmental stages. Beneficial symbionts can afford essential vitamins, amino acids and other exclusive novel enzymes to degrade cellulose materials present in the dung [49 - 51].

The gut microbiota of herbivore insect has been much explained by two kinds of the functions such as nutrient biosynthesis and the biomass deconstruction [43]. Gut microbes present in the xylaphagous and coprophagous insects involved in the cellulolytic material’s degradation, where cellulose exists in crystalline nature in the plant cell wall protected by a barrier containing lignin from degradation by insect gut digestive enzymes. Breakdown process of cellulose fibers into simpler sugar molecules were achieved by gut microbiota [52 - 56]. Xylose is a complex material generally present as an insoluble sugar in nature initially converted to xyitol, followed by the conversion of xylose in yeast fermentation process [57]. Yeasts varieties such as Candida tanzawaensis, Candida ambrosiae, Symbiotaphrina buchneri, Candida xestobii isolated from passalid beetles Odontotaeini disjunctus and Verres sternbergianus were proclaimed as potent xylose fermenting agents [23]. Lignin-cellulose bonds of lignocellulose were cleaved by insect gut fungal enzymes [58, 59].

Cellulose degradation was achieved successfully when the gut microbes such as Pseudomonas nitroreducens, Bacillus licheniformis, Rhizobium radiobacter, Microbacterium oxydans, Citrobacter freundii, Paracoccus sulfuroxidans, Cellulosimicrobium funkei of scarab dung beetles Holotrichia parallela and Euoniticellus intermedius were grown on cellulosic medium with filter paper alone acting as a sole carbon source [60,61]. Pectin degrading genes from γ-proteobacteria Gilliamella apicola was identified in honey bee where the pollen wall was primarily loosened by the degradative action of bacteria [62, 63]. Tannins, a major phenolic polymers produced by higher plants are harder to degrade by the insects. Gastrointestinal microbes like Streptococcus galactolyticus, Selenomonas ruminatium and Lonepinella koalarum were reported as a predominant bacterial species for the degradation of hydrolysable tannins [64]. Digestion of tannic acid was achieved by yeast species [39]. Thus, the numerous potent microbes accommodate in scarab beetle gut and aid in converting the incomplete diet into an easily digestible and vitamin rich source.

**Role of Scarab beetle gut microbes in metabolic processes**

Apart from digestive process, gut microbes are associated with various metabolic processes in the host for better the nutrient absorption. Gut bacteria mainly perform certain functions like acetogenesis, methanogenesis, hydro genesis, and reduction of sulfate along with the plant polymer degradation to reach a high degree of nutrient uptake by the host. As many fermenting bacteria present were in the hindgut of scarab larvae, massive concentrations of elusive fatty acids were seen due to natural anaerobic processes like methanogenesis [32]. Frass producing Enterobacteriaceae of Rhyncophorus ferrugineus acted as an anaerobe in 2,3-butanediol fermentative metabolism and when they combined with Lactococcus species, it led to the production of ethanol, acetone, lactate and CO2 [65]. Nitrogen volatilization and mineralization are the major ecological processes executed by dung beetles in association with its gut microbes. Ammonifier microbes present in dung beetles may also pave way for N-mineralization [9, 12, and 66]. Gut bacteria of termites can effectively fix nitrogen from waste material recycling and also directly from the atmosphere [67-69]. Many different microbes evidently produce several metabolites encompassing tiny organic molecules which trigger the host insects.

**Novel products from Scarab beetle gut microbes**

Insect gut microbial community is believed to produce metabolically prominent products which are beneficial to both insect and its symbionts. Digestion of cellulose in insects is proficient by the collaboration of some enzymes which form a cellulase complex. The important classes of cellulolytic enzymes from gut fungal and bacterial species are endoglucanases, 3-glucosidases, exoglucanases and cellulohydrolases [70 - 72]. Majority of cellulase producing microbes from termite gut, stem, and other Coleoptera species belong to Enterobacteriaceae family. Carboxymethylxylanase and xylanase enzyme producing bacterial species were confirmed by zymogram analysis [73]. Two major cellulose degrading enzymes, β-1, 4-endoglucanase and β-1, 4-endoxylanase of Paenibacillus strain were isolated from cotton bollworm gut [74]. Several novel xylanase enzymes from microbial consortia of insect guts with novel domains such as xylan polymer binding site and cleavage patterns were reported [75]. Insects armed with some fungal species like Piromonas communis, Orpinomyces sp. and Neocallichasmix frontalis were the producers of xylanases for efficient xylan degradation. Novel products from yeasts such as Pichia stipitis and gut bacterium like Bacillus halodurans, Ruminococcus albus, Bacillus sp. Strain KK-1 and Pseudomonas halolankits were also reported to be the producers of the xylanase from wood-feeding scarab beetles [23, 75].
Amphastin, a Gram-negative bacterium, was isolated from the hindgut of Scarab beetle through metagenomic analysis [88]. Amphastin was shown to possess antimicrobial activity against various Gram-positive and Gram-negative bacteria. Metabolites containing the secretion of immunological compounds are not constant and totally depend on the infection [101,102]. Streptomyces sp., a gut microbe from southern pine beetle Dendroctonus frontalis produced polyene mycosaminecin, a structurally novel bioactive compound, and frontalalides, another polycyclic tetramate macrolactam antibiotic compound [103,104]. The wasp Philanthus triangulum showing mutualism with gut bacteria. Streptomyces sp. was proved to produce small peptide like molecules and sceliprolactam, an antifungal agent which protected the beetle cocoon from fungal infections and also encouraged larval survival [105,106]. Coprisamide A and coprisamide B were the branched cyclic peptides isolated from the gut of dung beetle C. tripartitus. This finding proved that the gut microbes of dung beetle will be a promising source of many antimicrobial peptides. Coprisamides showed notable activity on quinone reductase induction [107]. Another metabolite of Streptomyces sp. was tripartilactam, a cyclobutane bearing tricyclic lactam identified in the brood ball of dung beetles. This tripartilactam metabolite acted as a Na+/K+ ATPase inhibitor [108]. In P. aphid Acrystosiphon pismum, heat tolerant symbiont Buchnera, a facultative bacterium was isolated when the aphid was subjected to stress [109] whereas cold tolerance was reported in four bark beetle varieties such as Ips graniicollis, Dendroctonus frontalis, Ips perroti and Ips pini [110]. In Drosophila melanogaster, its gut bacterium Lactobacillus plantarum showed diet-induced mating preference which paved a way to know about the advanced role of gut microbiota in hologenome concept [111]. From these findings, it was proven that gut microbes of insects can boost up insect fitness even in the pathogenic environment.

**Role of scarab gut microbes in insect immune system**

Insects hold an excellent innate immune system which protects them from various pathogens due to production of antimicrobial peptides and reactive oxygen species [86]. In response to various microbial infections, up-regulation of many immune-related genes occurring in insects resulted in the production of Antimicrobial peptides through Imd pathways [87-89]. Ferrandt et al. (2007). [90] Provided a detailed review on the amazing defensive mechanisms of the insects. Numerous types of antimicrobial peptides have been produced and characterized from Coleopteran insects like Zophobas atratus, Holotrichia diomphalia, StilPUophilus oryzae, Acacolepta luxuriosa and Allomyrina dichotoma. They were proved to be the producers of coleopterixin-like AMPs like Coleopterixin, rhinocerolin, holotrichin and acacoleptin [91-95]. Metagenomic analysis of Protactea brevisarsi sensu larvae was performed to identify immune-related genes of the beetle [96]. Hwang et al, (2009). [97] Reported the effective innate immunity of dung beetle Copris tripartitus when injecting the beetle larvae with lipopolysaccharides. Another novel product, a defensin-like peptide, coprisin was also isolated from dung beetle Copris tripartitus, which showed potential antimicrobial activities against some Gram-negative and Gram-positive bacteria like Staphylococcus aureus [98]. Antimicrobial proteins were discovered from the cocoon shell extract of lepidopteran insect B. mori against a variety of pathogenic microbes [99]. In this context, we intended to explore about the key role of gut microbes in the gut and how it influenced the insect immune system. In general, scarab beetles spend their whole lives in the soil containing dung and other undigested waste materials. Dung contain pathogenic microbes along with dung material from the gastrointestinal tract of mammals. Many metabolites comprising tiny organic molecules produced by gut microbes may help stimulate host cell immune system [100]. Apart from insect’s excellent defensive strategies, it is evidently proven by many researchers that gut microbiome also produces many smart molecules that stimulate host immunity at the time of a pathogenic attack. **CONCLUSION**

Coleopteran dung beetles are the most diverse group of insects responsible for various important ecological functions like recycling of recalcitrant plant polymers, soil enrichment etc. Gut microbes from scarab beetles showed good potent application for dung, wood and other waste material’s degradation. Degradation of cellulose and lignocellulosic material was carried out by the production of novel enzymes/peptides by gut symbionts. This scarab hindgut region was well suited for the colonization of gut microbes where they produced abundant digestive enzymes and so it was also termed as bioreactor. Cellulose and lignocelluloses degradation is the most important process in biofuel/bioethanol production. Potent tannic acid degrading microbes identified from dung beetle gut showed efficient role in many industries like wine clarification, effluent treatment, textile industries etc. Many metabolites which induce host immune system were also discovered from insect gut microbiome. So, proper characterization of gut microbes from Scarab beetle, producing novel enzymes are needed for various industrial purposes. This review will open up a new room of thoughts on the diversified role of Scarab gut microbes with reference to many industrial applications.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.
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