

Isolation and Identification of Microbial Load in the Gut and Faeces of Millipede *Spinotarsus colosseus*

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Abstract: This investigation illustrates the fungal and bacterial population from the alimentary canal and fecal pellets of the Millipede *Spinotarsus colosseus*. This microbial load in their gut makes Millipede efficient decomposers of detritus. The bacterial and fungal community obtained from the gut extract also exists in the fecal pellets which are considered as the compost. It was identified that the total of five fungi species from the gut extract, out of which three species existed in fecal material also. Seven species of bacteria were isolated from the gut and five of them prevail in the excrement. Four fungal populations come under the division Ascomycota and one belongs to Zygomycota. Of the total heterotrophic bacteria isolated, five are Gram negative and two are Gram positive. The millipedes and its gut microbes are in symbiotic relationship with each other thereby enhancing the degradation process of leaf litter and detritus. Hence the present study reveals that the fungal load gradually decreased from gut content to the excrement.

Key words: *Spinotarsus colosseus* • Fungi • Bacteria • Detritivores • Ascomycota Zygomycota

INTRODUCTION

Organic farming relies heavily on the natural breakdown of organic matter, by using one of the most vital techniques is called composting, to replace nutrients taken from the soil by previous crops. Nowadays we experience a rapid urbanization and industrialization which have led to the abundant accumulation of waste materials. In order to degrade the accumulated waste, the widely practiced traditional method is compost production. The well known composting method is vermicomposting effected by the lowly invertebrate known to man ever since are the Earthworms. It is the most suitable and eco-friendly method of solid waste management when compared to land reclamation, incineration etc. but the composting is also done by another macro arthropod known as Millipedes. They are commonly called as thousand legged animals. Literally they are slow movers when compared to centipedes. Similar to earthworms, they also involve in the humification and nutrient enrichment of soil. They dwell under the top soil and they are efficient burrowers. They are considered as the bio-indicators of the environment, because the appearance and activity of millipedes are abundant in the rainy season and humid conditions

whereas the increase in temperature and lack of rainfall results in decrease of millipedes.

They are representatives of the soil macro fauna that live in the deciduous forest litter and play an important role in the decomposition processes of decaying plant material. The food source of the millipedes is the forest litter. The digestion of leaf litter and decaying matter is mainly due to the microbial load residing in the gut of the millipedes. The gut of millipedes may represent a reservoir of bacterial and fungal species. The intestinal microbial community plays a significant role in the breakdown of the plant polymers. Millipedes also enrich the soil nutrition as earthworms. Millipedes, being a well know macro arthropod, the process of milicomposting is not practiced when compared to vermicomposting. Though millipedes are macro detrivores and saprophagus, the involvement of microbial population dwelling in the gut plays a vital role in degradation of solid waste. The gut bacteria of Millipedes play a predominant role in the degradation of litter materials they consume and the gut microbiota are able to synthesize many enzymes for the digestion and assimilation of plant materials. The presence of bacterial species belonging to *Klebsiella*, *Sarcina*, *Bacillus* and *Corynebacterium* were also reported [1].

One of the Millipedes *Xenobolus carnifex* is considered as the household pest because it widely affects the thatched roofing materials made out of the reed *Aristida sativa*. The microbiota found in the gut helps in easier digestion of cellulose and lignin material present in the feed material and thereby determining the rate and efficiency of utilization of these materials [2]. Millipede gut provide unique ecological niche for many microorganisms, which are unable to survive outside [3]. The organic matter fed by the Millipedes are processed by bacteria and fungi colonised in their gut [4, 5]. Many microbes are unique to the intestine of millipedes are not inhabitants in leaf litter or soil [6]. Some microbes exist as endosymbionts with millipedes and transmitted through eggs to the next generation [7].

In the present investigation the bacterial and fungal populations were isolated from the gut and faeces of the millipede *Spinotarsuscolosseus*. The study reveals that, bacteria steadily increased from gut content to feces but filamentous fungi were substantially decreased from gut to feces.

MATERIALS AND METHODS

Adult millipede species, *Spinotarsuscolosseus* were collected from Palamedu village (S|11° 46' |N|78° 53'). It has an average elevation of 227 meters (745 feet) with moderate monsoon climatic condition located near Madurai district. These millipedes were acclimatized to the laboratory condition by providing sterile food and maintained at 28° ± 2°C for 10 days.

The millipede *S. colosseus* was anesthetized by using chloroform in a cotton swab and surface sterilized with 1% mercuric chloride and ethanol for 2 minutes, again washed with distilled water. The body cavity was opened ventrally and flooded with sterile Ringer's solution. The gut of the was dissected out aseptically and homogenized along with the same solution. The millipede gut extract was serially diluted and dilution plates were done for isolation of bacteria and fungi. The serially diluted sample was loaded on Potato Dextrose Agar (PDA) along with the incorporation of antibiotic Streptomycin. The plates were incubated at 30°C ± 2°C for 4 days. After the growth of fungal colonies, they were again streaked and incubated for 48 to 72 hours for the isolation of pure culture. The morphological identification was done by slide culture method. The fungal growth obtained was stained with lactophenol cotton blue stain and were examined under the microscope.

Similarly, the dilution samples were again loaded on Nutrient agar medium and incubated at 37°C for 24 hours for the growth of bacterial population. From the pure culture the isolated colony were performed for biochemical test for the identification of bacteria according to Bergey's manual of systemic Bacteriology [8].

RESULTS

The wet weight of the millipede gut weighed about 1.853±0.5 gram and its pH measured about 5.7. The colony forming unit of bacterial and fungal communities was calculated separately from both gut content and excrement. The total bacterial count of the gut and excrement was estimated as 3.4 x 10⁶ and 2.8 x 10⁶ CFU/ml respectively, the fungal count of the gut and excrement was calculated to be 5.7 x 10⁵ and 4.4 x 10⁵ CFU/ml respectively. Seven species of gut bacteria were isolated from the millipede *S. colosseus* and five of them were repopulated also in the fecal material. *Citrobacter freundii*, *Klebsellia pneumonia*, *Erwinia* species, *Cornebacterium* species, *Bacillus subtilus*, *Escherichia coli* and *Alcaligenes faecalis* were isolated from the gut content. From these the following species.

Table 1: Characteristics of gut bacteria of Millipede *S. colosseus*

Biochemical analysis	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Gram's staining	-	-	-	-
Morphology	R	R	R	R
Motility	+	-	+	+
Catalase	+	+	+	+
Indole production	-	-	+	-
Methyl red	+	+	-	-
Voges proskauer	-	-	+	-
Citrate utilization	+	+	-	+
H ₂ S production	+	-	+	-
Starch hydrolysis	-	+	-	-
Gelatin hydrolysis	-	-	+	-
Urease	+	-	-	-
Oxidase	-	-	-	+
Glucose	+	+	+	+
Lactose	+	+	+	-
Sucrose	+	-	+	+
Mannitol	+	+	+	-
Arabinose	+	+	+	-
Xylose	+	+	+	-
Raffinose	-	+	-	-
Phenylalanine deaminase	-	-	-	-
Identified as	<i>C. freundii</i>	<i>K. pneumoniae</i>	<i>Erwinia species</i>	<i>A. faecalis</i>

Table 1:

Biochemical analysis	Isolate 5	Isolate 6	Isolate 7
Gram's staining	-	+	+
Morphology	R	R	R
Motility	+	-	-
Catalase	+	+	+
Indole production	+	-	-
Methyl red	+	-	-
Voges Proskauer	-	-	-
Citrate utilization	-	-	-
H ₂ S production	-	-	-
Starch hydrolysis	-	+	+
Gelatin hydrolysis	-	-	-
Urease	-	-	-
Oxidase	-	-	-
Glucose	+	+	+
Lactose	+	-	-
Sucrose	+	-	-
Mannitol	+	-	-
Arabinose	+	+	+
Xylose	-	-	-
Raffinose	-	+	+
Phenylalanine deaminase	-	-	+
Identified as	<i>E. coli</i>	<i>B. subtilis</i>	<i>Corneybacterium</i> species

Table 2: Fungal population found in the gut and faecal material of millipede *S. colosseus*

Division	Fungal population
Ascomycota	<i>Aspergillus fumigatus</i>
Ascomycota	<i>Aspergillus niger</i>
Ascomycota	<i>Aspergillus tamarii</i>
Ascomycota	<i>Aspergillus flavus</i>
zygomycota	<i>Absidia corymbifera</i>

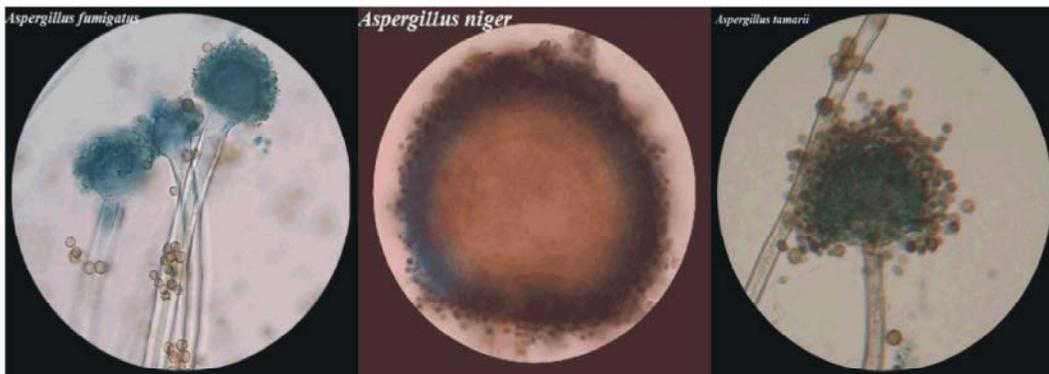


Fig. 1: *Aspergillus fumigatus*

Fig. 2: *Aspergillus niger*

Fig. 3: *Aspergillus tamarii*

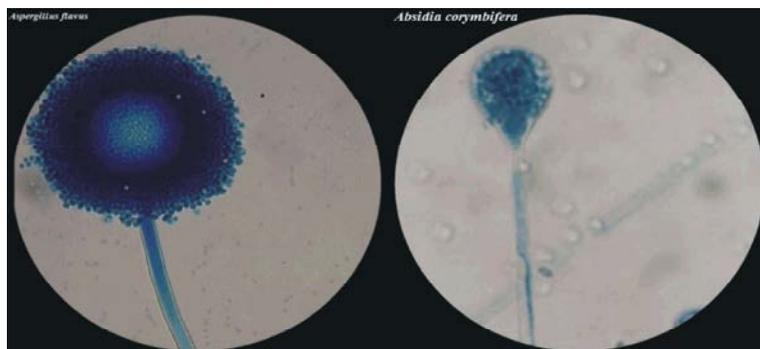


Fig. 4: *Aspergillus flavus*

Fig. 5: *Absidia corymbifera*

C. freundii, *K. pneumonia*, *Erwinia* species, *Corneybacterium* species and *Alcaligenes faecalis* were resided in the fecal material. The fungal population identified from the gut extract were, *Aspergillus fumigates*, *Aspergillus niger*, *Aspergillus tamari*, *Aspergillus flavus* and *Absidia corymbifera*. From the above mentioned species, *A. fumigates* and *A. corymbifera* were reloaded in the excrement.

DISCUSSION

Coprophyagy is a common phenomenon in this animal group which feed on their own fecal pellets and exuvia. Some tend to be mycophagy, feeding on the fungal growth. Out of the total feed intake, millipedes utilizes only one tenth of the food. 90% of the ingested leaf litter is shipped and relocated in the fecal pellets from gut passage by the myriapods. The transformation of leaf litter into faeces by the saprophagumacroarthropods can influence the process of digestion and decomposition by several ways [9]. It has been authenticated that the activity of bacteria and fungi plays a preceding role in the process of decomposition which recalibrates the physical and chemical composition of the leaf litter engrossed by the millipede [10, 11]. Almost half of the diplopod populations are speculated to feed on fungi, because the ascospore releasing structures from the fungi are responsible for the assimilation of calcium by the diplopods. They employ these high calcium and metamorphose them into calcium oxalate for the architecture of its tergites [12]. This results in dismounting of fungal population from the voyage of leaf litter to fecal pellet. This proves a strong documentation for the subsidence of fungal population. An intensified range between 10 and 100 folds were recorded in bacterial population, whereas the range for fungal population decreased upto 3.5 folds from feed to fecal pellet [13]. The consumption of bacteria paved way for the animals in higher assimilation efficiency, while the consumption of fungi leads to the amplified rate of CO₂ waste.

The fungal species *A. niger* build up its cell wall by the chitin. Hence the millipede utilizes the serviceability of these fungi for the digestion of chitin [14]. Similarly, the fungi *A. corymbifera* utilizes the cellulose. The species *Pseudallaescheria boydii* and *A. fumigates* were found abundant in vermicompost which actively involved in Keratinolytic activity [15].

Pseudomonas putida and *Pseudomonas stutzeri* were likely to be found abundant in the fecal pellets of millipede *Pachyiulus flavipes* when compared with its gut microflora [16]. The fecal pellet of the millipede species

A. magna serves as a reservoir of several bacterial groups such as *Methylobacterium populi*, *Novosphingobiumsubarcticum* under alpha proteobacteria, the species *Cupriavidus basilensis*, *Pseudomonas putida* group under beta proteobacteria, gamma proteobacteria includes the species *Pantoea agglomerans*, *Pseudoxanthomonas koreensis*, *Klebsiella pneumonia*, *Citrobacter amalonaticus* and bacilli group encompass *Bacillus licheniformis*, *Bacillus arsenicus* and *Bacillus subtilis* [6]. The gut of the millipede serves as a vast commorancy of microflora which also includes methanogenic bacterial diversity found in the species of Julida, Spirobolida and Spirostreptida [17]. Almost all the millipedes lodging in tropical region tend to produce methane, while the millipedes belonging to temperate climates do not [18]. The species of European millipede belonging to Julida were detected to produce methane but the other groups such as Polydesmida and Glomerida are unskilled for methane production [19].

The gut and faeces of these diplopods serves as a potential habitat for bacteria which enhanced the bacterial growth [13, 20]. *A. newtoni* is observed as a host for the *Bacillus thuringiensis* [21]. Previous reports reveal that the growth and multiplication of the microflora is maximum in the hindgut when compared to midgut. The reason behind this is that the midgut fluid possesses the antimicrobial activity. The midgut is the site of degradation of cellulose and hemicelluloses whereas the midgut is the site of degradation of pectin. The hindgut is the major site of bacterial and fungal proliferation [22].

CONCLUSION

Bano *et al.* [23] recoded that the total colony forming unit of 153.5×10^4 CFU/g dry wt for the species *Jonespeltis splendidus*. Similarly, *Glomeris marignata* recorded 23.4×10^7 CFU/g dry wt [13] 22.8×10^8 CFU/g dry wt for the same species by Ineson and Anderson [24]. *Ommatoiulus sabulosus* counted 3.84×10^6 CFU/ g dry wt [25]. *Xenobolous carnifex* counted 1.4×10^8 CFU/g dry wt by Alagesan *et al.* [2] and *Arthospharea magna*, *Aulocolobolusnewtoni*, *Spinotarsuscolosseus* recorded 3.8×10^{11} CFU/g dry wt, 3.1×10^{11} CFU/g dry wt, 4.7×10^{11} CFU/g dry wt, respectively by Ramanathan and Alagesan, 2012. The millipede, *Glomeris marginata* serves as the host for bacterial population like *Pseudomonas alcaligenes*, *Klebsiella pneumoniae* [26] the following bacterial community such as *Klebsiella*, *Sarcina*, *Bacillus* and *Corynebacterium* species exist in the gut of the millipede *Schizophyllum sabulosum* [1]. The

bacteria *Escherichia coli*, *Enterobacter agglomerans*, *Klebsiella*, *Pseudomonas fluorescence*, *Sarcina* abodes in the species *Ommatoiulus sabulosus* [25]. The bacterial community *Proteus mirabilis* and *Citrobacter freundii* make their dwelling place in the gut of the millipede *Xenobolus carnifex* [2].

The bacterial communities such as actinomycetes, heterotrophic bacteria, phosphate solubilizing bacteria and rhizobia tend to escalate their population from leaf litter to gut, but the filamentous fungi and yeast population tends to decrease because the fungal population plays a decisive role in making availability of vitamins, essential amino acids, etc. which could not be gained by the millipedes especially for desert dwelling species [27, 28]. Hence, the present study affirms that a total of 7 species of bacteria and 5 species of fungi were isolated from their gut content and among them, 5 bacteria and 2 fungal species were reloaded in the excrement of the millipede *S. colosseus*. Thus, with the aid of the microflora, millipede acts as efficient decomposers of organic matter.

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