4 Intestinal Microbiota of Millipedes

Boris A. Byzov

4.1 Introduction

Soil millipedes (Diplopoda) possess a specific gut microbiota that differs from microbial communities in soil and leaf litter. A diverse microbiota has been found by the dilution plating in the gut with gamma proteobacteria, actinobacteria and yeasts most abundant. Microscope studies also revealed a variety of morphotypes of bacteria and yeasts attached to the gut walls. Evidence is discussed that the millipedes have symbiotic associations with microorganisms that have particular functional roles for the host animal. The possible functions of gut microorganisms are the participation in the digestive processes, the maintaining of microbial community in a steady state, in vivo production of methane. The "killing effect" (a lytic process) is discussed as one of the possible mechanism for the digestion of microorganisms by millipedes. It is most likely that the saprophagous millipedes do not harbour lignocellulose-degrading microorganisms; most likely, they feed on microorganisms and use microbial enzymes to digest recalcitrant molecules.

Scientific interest in millipedes is largely related to their participation in organic matter decomposition and nutrient cycling. These processes are mediated in soil by activities such as fragmentation of leaves, stimulation of microbial growth, and, subsequently, deposition of faecal pellets (Hanlon 1981). There are few habitats in which millipedes are responsible for ingesting more than 5–10% of the annual leaf litter fall; however, when earthworms are scarce, millipedes may occur at densities of several hundred/m^2 and consume 25% of the litter fall (Hopkin and Read 1992). Although millipedes (and other saprophages altogether) are directly responsible for less than 10% of chemical decomposition, their feeding activities are important in stimulating soil microorganisms, which carry out 90% of chemical breakdown (Anderson and Bignell 1980). This aspect

Boris A. Byzov: Department of Soil Biology, Faculty of Soil Science, Moscow Lomonosov State University, GSP-2, 119992, Leninskie gory, Moscow, Russia, E-mail: boris.byzov@ps.msu.ru

Soil Biology, Volume 6
Intestinal Microorganisms of Soil Invertebrates
H. König, A. Varma (Eds.)

of microbial-millipede interactions is vital in studying the importance of invertebrates in food web functioning.

The nature of the gut microbiota of millipedes is probably less important in terms of ecosystem related processes. However, the knowledge of species diversity of gut symbionts of soil invertebrates and their functional importance for the host animals are scare (except for termites) although millipedes, and many other soil invertebrates, have a specific digestive system that involves interaction with gut-associated microbes.

In this chapter, an attempt is made to present and discuss the data on morphological and taxonomic diversity of the millipede intestinal microorganisms and their possible functions.

4.2 Structure and Function of the Digestive Tract

The foregut of millipedes is poorly populated by microorganisms. The midgut represents the absorptive surface and continuously secretes the semipermeable peritrophic membrane. The peritrophic membrane has a highly ordered net-like structure composed of microfibres. In *Glomeris marginata* the fibres are arranged in uniformly sized squares of 0.1 µm (Martin and Kirkham 1989), in *Pachyiulus flavipes*, 0.05–0.1 µm (Byzov et al. 1993c), and in *Glomeris connexa,* 0.15–0.2 µm in size (Byzov et al. 1996). The membrane encloses the solid food materials preventing their direct contact with the midgut epithelium. There is no direct evidence that the peritrophic membrane represents a barrier to microorganisms; however, the small size of holes in the membrane would also prevent access to the midgut epithelium by the majority of microorganisms in the gut contents. The membrane is an envelope composed of chitin, protein and mucopolysaccharides. In electron microscopic studies, the membrane first appears amongst the microvilli or at their tips. Chitin appears at the bases of the microvilli along the whole length of the midgut. Protein is added to the chitin from the microvilli as the peritrophic membrane moves along the microvilli to the lumen. The completed peritrophic membrane extends around individual items in the gut contents as well as forming a multilayered envelope. The peritrophic membrane is continuously secreted, and moves towards the hindgut. More commonly, the membrane is broken up in the hindgut by muscular contractions or spiny projections of the cuticle. Disruption of the peritrophic membrane may be of importance in allowing ingested organisms access to colonization sites on the hindgut cuticle. It was also suggested that the disruption of the membrane permitted some parasites to reach the mesenteric epithelium by forward migration along

the ectoperitrophic space. The functions of the peritrophic membrane are not fully known, but its most obvious purpose is to protect the absorptive surfaces in the absence of mucus secretion (Bignell 1984; Hopkin and Read 1992). The semi-permeable structure of the membrane makes it probable that digestion is sequenced radially, with partial degradation of macromolecules only taking place in the endoperitrophic space. The hydrolysis to dimers and oligomers occurs in the ectoperitrophic space separating the membrane and the midgut epithelium, and some terminal digestion occurs intracellularly or mediated by enzymes bound to the apical cell membrane (Hopkin and Read 1992).

The cuticle-lined hindgut is strongly developed and bears both flat cuticular surfaces and ornaments such as spines of various shapes, which provide sites for microbial colonization. In *Orthoporus ornatus,* the cuticle of the hindgut is formed into numerous projections, each of which has central depression in which bacteria may reside (Crawford et al. 1983). Malpighian tubules open at a junction point between midgut and hindgut. Their function is to deliver to both the midgut and the hindgut a fluid containing many of the haemolymph constituents at concentrations proportional to their concentrations in the haemolymph. The secretion of the Malpighian tubules constitutes not only a nutrient broth of balanced composition but also an effective buffer solution. In physiological terms, this may imply that the hindgut of millipedes offers a suitable site for microbial colonization. The study made by scanning electron microscope confirmed that an abundant and diverse microflora occurred in this location. Additional fluid input to the gut takes place via salivary glands and accompanies ingestion of food (Bignell 1984).

The Malpighian tubules are also involved in excretion of nitrogenous wastes. In millipedes, two forms of nitrogenous wastes predominate: ammonia (which has to be excreted rapidly) and uric acid (the stored form). Urea is detected less frequently. The proportion of ammonia and uric acid varies between species. For example, in *Cylindroiulus londinensis,* only 20% of non-protein was ammonia whereas 70% was uric acid. Of the total non-protein nitrogen in the faeces of *Glomeris marginata*, 40% was ammonia and 33% was uric acid (Hopkin and Read 1992). However, Anderson and Ineson (1983) were unable to detect any uric acid in the faeces of *Glomeris marginata* although the animals themselves contained 2.5% uric acid by weight. The authors have suggested that uric acid might form a reserve of nitrogen for use when food contains insufficient amount of the element. The release of the uric acid in the hindgut may also provide source of nitrogen for gut microflora (Byzov et al. 1993b).

For more details related to the anatomic structure and functions of the millipede intestine, see Hopkin and Read (1992).

4.3 Physiological Conditions in the Gut

The redox potentials, determined with a platinum and calomel electrode combination, in the midgut of *Glomeris marginata* vary between +267 and +307 mV and in its hindgut between +167 and +277 mV. Mean redox potentials in the lumen of the digestive tract are +232 mV in the midgut and +204 mV in the hindgut (Bignell 1984). Therefore, the gut environment is not an anaerobic but has moderate reduced conditions (microaerophilic). The pH of the contents of the lumen of the midgut of millipedes is rarely more than one unit either side of neutrality (pH 6 to 8) (Bignell 1984). Byzov et al. (1998) found the mean pH of the midgut fluid of the diplopod *Pachyiulus flavipes* to be 5.6.

Concentrations of glucose were found to be 6.0 mmol/l (1.1 g/l) in the midgut fluid of the diplopod *Pachyiulus flavipes,* whereas in the hindgut extract it was 12.7 mmol/l (2.3 g/l). In the midgut fluid of *Rossiulus kessleri* the concentration of glucose was 7.1 (1.28 g/l) mmol/l (Byzov 2003). Such rather high concentrations of glucose are comparable to that of nutrient media and may indicate that active hydrolysis of carbohydrates takes place in the millipede digestive tracts.

Hopkin and Read (1992) have reviewed the data on enzymatic activities of the millipede digestive tracts. In many millipedes, enzymes have been found that are capable of digesting lipids, proteins, and simple carbohydrates (Nunez and Crawford 1976; Neuhauser and Hartenstein 1976; Marcuzzi and Turchetto-Lafisca 1977; Kaplan and Hartenstein 1978; Neuchauser et al. 1978). However, there is still no strong evidence that millipedes themselves are able to digest more refractory components of leaves. For example, Neuchauser et al. (1978) showed that *Oxidus gracilis* was unable to degrade [^{14}C]-lignin. In contrast, *Pseudopolydesmus serratus* was able to degrade components of ligneous compounds (Neuhauser and Hartenstein 1976). Similarly, there is conflicting evidence regarding cellulose breakdown. It was found that gut extracts of *Pachyiulus foetidissimus* hydrolysed cellulose (Striganova 1970) and those of *Polydesmus angustus* were able to break down cellulose, hemicelluloses, and pectin (Beck and Friebe 1981). However, no cellulase was found in millipedes from families Glomeridae and Polydesmidae (Nielsen 1962; Marcuzzi and Turchetto 1975).

Cellulases in these gut extracts were probably derived from gut inhabiting microorganisms (Hopkin and Read 1992) or from enzymes ingested with food (fungi), as known for the digestive process in insects. In the giant desert millipede *Orthoporus ornatus* and the slate millipede *Comanchelus* sp., enzyme assays indicated that most cellulose and hemicellulose degradation occurred in the midgut, whereas the hindgut was important site for pectin degradation. Hemicellulase and β-glucosidase in both species

and C_x-cellulase and pectinase in *O. ornatus* were of possible microbial origin. This was confirmed by the experiment with millipedes whose gut microbiota was reduced by antibiotic treatment and starvation. The treated animals assimilated less ^{14}C-cellulose and voided more ^{14}C in faeces (Taylor 1982). Finally, there is no convincing report of the production of C_1-cellulase by soil invertebrates, although β-gluconases (C_x-cellulase) and cellobiase of animal origin have been found. Most of the data on C_1-cellulase production by soil animals were obtained in studies where microbial activity (especially activity of symbionts) had not been excluded.

Chitinase activity was found in some millipedes suggesting that digestion of fungi could take place in the gut (Nielsen 1962; Marcuzzi and Turchetto 1975).

4.4 Microscope Studies of Intestinal Microbiota

4.4.1 Bacteria

In scanning electron microscopy studies by Bignell (1984), actinomycete-like filaments were found to be end-on attached to flat cuticular surfaces of *Cylindroiulus* sp. Actinomycete-like epibionts (diameter of hyphae 1.2–1.3 µm) were also found in the hindgut of *Tachypodiulus sp.* More recently, it was found that the inner surface of the intestinal walls of the millipedes *Chromatoiulus rossicus* and *Glomeris connexa* is sparsely colonized by bacteria of different morphotypes. Very small cocci (< 0.2 µm), rod-shaped bacteria, small rod-shaped slightly curved bacteria, rod shaped bacteria covered with the slime, V-type forms (presumably coryneforms), cocci or short rods forming short chains or mycelium-like nets. The latter type represents the most frequent bacterial form in all gut parts. On the endoperitrophic surface of the peritrophic membrane bacterial cells can also be seen. The surfaces of the gut content were colonized mainly by filamentous bacteria of two types, presumably belonging to coryneform bacteria and actinomycetes. The coryneform bacteria had a pseudomycelium with typical branching cells, which disintegrated into single cells. The actinomycetes had well branching filaments no more than 1.0 µm in diameter. The organic particles were also colonized by *Vibrio*-like bacteria and large rod shaped bacteria. In fresh excrement, rod-shaped bacteria of two types were observed. Some of them were partially lysed (Byzov et al. 1996a). In *Pachyiulus flavipes,* the actinomycete mycelium (0.5 µm in diameter) was not found on the midgut wall but was abundant on the hindgut wall. It occupied the inner surface of peritrophic membrane in the midgut, and

its remnants in the hindgut. Streptomycete-like hyphae produced spore chains indicating that the actinomycete can complete its life cycle in the gut. The numbers of bacteria in micro-colonies varied from several hundreds to thousands. The total number was higher in the hindgut (Byzov et al. 1993c).

That the millipede guts represent a suitable place for actinomycete growth has been also shown by Polyanskaya et al. (1996). With the aid of luminescent microscopy, they found an orange luminescence emitted by the hyphae of *Streptomyces olivocinereus* after spores were fed to the millipede *Pachyiulus flavipes*.

The biometric analysis of 1953 microphotographs of bacterial cells associated with the guts or fresh faeces of millipedes *Glomeris connexa*, *Leptoiulus polonicus, Megaphyllum projectun* and *M. rossicum,* obtained by scanning electron microscope, revealed 24 morphotypes of bacteria of different size classes from very small cocci to long rods. It has been shown that their average size is 0.66 μm in diameter, 1.35 μm in length, and 0.6 μm^3 in volume. The average diameter, the length, and volume of the bacterial cells inhabiting native soil amended with organic substrates (glucose, starch, cellulose) (from 2645 micrographs) were found to be 32%, 16% and 36% larger, respectively. The authors have suggested that in the digestive tract of soil animals activation of the bacteria took place resulting in a higher metabolic activity of the cells and in a selection of smaller cells (Guzev and Zvyagintsev 2003).

Very limited data exist for the direct counts of the intestinal bacteria. With the aid of a luminescent microscopy, Polyanskaya et al. (1996) have found extremely high numbers of prokaryotic unicellular organisms in the midgut, hindgut and fresh faeces of the diplopod *Pachyiulus flavipes*, 1.0×10^{11}, 2.5×10^{11} and 1.0×10^{11} cells/g dry weight, respectively. The length of actinomycete hyphae was 5, 22 and 15 m/g, respectively. The animals were fed with sterile crystalline cellulose for 7 days, allowing appreciable voiding of the gut from transient microbiota. Thus, the counted bacteria were considered indigenous to the millipede. Similar results were obtained by Cazemier et al. (1997) who counted DAPI-stained bacteria in the gut preparations of *Chicobolus sp.* The bacterial counts were 1.7, 1.4 and 15×10^9 cells/ml gut in the foregut, the midgut and the hindgut, respectively, which is comparable to the numbers in *P. flavipes* (dry weight of its gut is about 10 mg) and the volume of the gut of *Chicobolus sp.* was found to be ca. 2 ml (Cazemier et al. 1997). However, the latter authors did not found actinomycete hyphae. Tret'yakova et al. (1996) have found 4.3×10^{10} bacterial cells per g dry gut of *Pachyiulus flavipes* fed broad leaves litter that corresponded to ca. 10^8 /gut.

Thus, the millipede guts are highly populated with diverse morphotypes of bacteria both unicellular and mycelial. The facts that they either were

isolated from the pre-washed gut tissue or they were visualized on the intact gut walls (end-on attached to the walls) let us to consider the bacteria indigenous to the animals.

4.4.2 Yeasts

Scanning electron microscopy of washed gut tissue of millipedes has been used to investigate the distribution of yeasts in *Glomeris connexa*, *Leptoiulus polonicus* and *Megaphyllum projectum*. It has been demonstrated that yeasts mainly colonize the hindgut of freshly collected diplopod with densities of about 10^3 cells/mm^2 (10^4 cells/gut). Only a few cells were found in the midgut (Byzov et al. 1993b).

4.4.3 Mycelial Fungi

No native fungal hyphae have been observed on the gut walls of the millipedes *Pachyiulus flavipes* (Byzov et al. 1993c) and *Glomeris connexa*, and *Chromatoiulus rossicus* (Byzov et al. 1996), suggesting that they are destroyed by the digestion.

4.5 Taxonomic Studies of Intestinal Microbiota

Hopkin and Read (1992) pointed out that, although it is generally accepted that microorganisms are of vital importance in digestion, there is no evidence that millipedes possess a permanent symbiotic microflora similar to that of termites. This review will demonstrate, however, that there are symbiotic interactions between microorganisms and millipedes. To prove this it is necessary to not only demonstrate the specificity of the gut microbes but also show their functional importance for the host as it is was shown for termites.

The basic context for this discussion is that the gut of millipedes provides an ideal environment for microorganisms. The lumen is protected from the vagaries of the outside environment, is permanently moist, is buffered to fairly constant pH and redox potential, and its contents are mixed thoroughly by muscular action (Bignell 1984).

A wide range of microorganisms has been isolated by the dilution plate method from the gut of millipedes. The taxonomic composition of bacteria and yeasts inhabiting the millipede guts are shown in Tables 4.1 and 4.2.

Table 4.1. Taxonomic composition of the dominant bacteria isolated from or detected[a] in the millipede guts and fresh faeces

Group/genus/species	Host	Source of isolation	Reference
Gamma proteobacteria: *Klebsiella* sp.	*Schizophyllum sabulosum* var. *rubripes*	Gut content	Baleux and Vivares (1974)
Actinobacteria: *Corynebacterium* sp.			
Clostridia: *Sarcina* sp.			
Bacilli: *Bacillus* sp.			
Actinobacteria: *Promicromonospora*-type bacteria	*Leptoilulus proximus, Chromatoiulus projectus, Cylindroiulus luridus. C. boleti, Unciger foetidus*	Fresh faeces	Dzingov et al. (1982)
Actinobacteria: *Promicromonospora enterophila*	*Chromatoiulus projectus*	Fresh faeces	Jager et al. (1983)
Actinobacteria: *Promicromonospora* sp.	*Leptoiulus proximus, Cylindroiulus luridus, C. boleti, Unciger foetidus*	Gut content	Szabó et al. (1983)
Gamma proteobacteria: *Klebsiella* spp., *Pseudomonas* spp.	*Chromatoiulus projectus*	Gut	Contreras (1985)
Actinobacteria: *Micrococcus* spp.			
Gamma proteobacteria: *Klebsiella* spp., *Enterobacter* sp.	*Chromatoiulus projectus, Cylindroiulus boleti*	Gut content	Marialigeti et al. (1985)
Actinobacteria: *Promicromonospora enterophila, Micrococcus* spp., *Nocardia* sp.			
Bacilli: *Bacillus* spp.			
Unidentified nocardioform actinomycetes	*Glomeris hexasticha*	Gut, fresh faeces	Szabó et al. (1985); Szabó et al. (1987); Chu et al. (1987)

Table 4.1. (continued)

Group/genus/species	Host	Source of isolation	Reference
Gamma proteobacteria: *Klebsiella pneumonia, Enterobacter cloacae, Plesiomonas* sp.	*Pachyiulus flavipes*	Intestinal walls	Byzov et al. (1993); Tret'yakova et al. (1996); Nguyen Duc TL et al. (1996)
Actinobacteria: *Promicromonospora citrea, Streptomyces chrysomallus, S. candidus, Streptoverticillium* spp., *Micromonospora* spp.			
Bacilli: *Bacillus* spp.			
Gamma proteobacteria: *Enterobacter cloacae*	*Pachyiulus flavipes*	Peritrophic membrane	Byzov et al. (1993); Tret'yakova et al. (1996); Nguyen Duc TL et al. (1996)
Actinobacteria: *Streptomyces badius, S. fluorescens, S. xanthocidicus, S. filamentosus*			
Gamma proteobacteria: *Pseudomonas* sp.	*Pachyiulus flavipes*	Gut content	Byzov et al. (1993); Tret'yakova et al. (1996); Nguyen Duc TL et al. (1996)
Actinobacteria: *Promicromonospora* sp., *Rhodococcus* sp., *Streptomyces carpaticus, S. griseolus, S. pseudogriseolus, S. paucidiastaticus, S. felleus, S. chrysomallus, S. aburaviensis, S. nitrosporeus, S. globisporus, S. pluricolorescens, S. longisporoflavus, S. echinatus, Streptosporangium albidum, Micromonospora* spp., *Actinomadura* spp.			

Table 4.1. (continued)

Group/genus/species	Host	Source of isolation	Reference
Bacilli: *Bacillus* spp.			
Flavobacteria: *Flavobacterium* sp.,			
Gamma proteobacteria: *Klebsiella* sp., *Enterobacter* sp., *Escherichia* sp., *Plesiomonas* sp., *Vibrio* sp., *Erwinia* sp., *Azotobacter* sp., *Pseudomonas* spp.	*Glomeris connexa*, *Chromatoiulus rossicus*	Intestinal walls	Byzov et al. (1996)
Alpha proteobacteria: *Beijerinckia* sp.			
Actinobacteria: *Mycobacterium* sp., *Rhodococcus* sp., *Promicromonospora-Oerskovia*			
Flavobacteria: *Flavobacterium* spp.			
Unidentified methanogens	*Chicobolus* sp.	Hindgut	Hackstein and Stumm (1994)
Free methanogenic bacteria; ciliates with intracellular methanogens	*Orthoporus* sp., *Rhapidostreptus virgator*; two unidentified species		
Free methanogenic bacteria	two unidentified species		
Gammaproteobacteria: *Erwinia* sp., *Pseudomonas* spp., *Azotobacter* sp.	*Glomeris connexa*, *Chromatoiulus rossicus*	Gut content	Byzov et al. (1996)
Alphaproteobacteria: *Beijerinckia* sp.			
Actinobacteria: *Streptomyces fimicarius, S. griseolus, S. wedmorensis, Promicromonospora-Oerskovia*			
Flavobacteria: *Flavobacterium* spp.			
Bacilli: *Bacillus megaterium, B. cereus, B. licheniformis*			
Flexibacteriaceae: *Cytophaga* sp.			

Table 4.1. (continued)

Group/genus/species	Host	Source of isolation	Reference
unidentified Myxobacteria			
Gammaproteobacteria: *Escherichia coli, Enterobacter agglomerans, Klebsiella* sp., *Pseudomonas fluorescens*	*Ommatoiulus sabulosus*	Gut content	Jarosz and Kania (2000)
Clostridia: *Sarcina* sp.			
Bacilli: *Bacillus* sp.			
Actinobacteria: *Micrococcus* sp.			
Six actinomycete and two fungal species			
Bacilli: *Bacillus brevis*	*Glomeris* sp.	Hindgut	Gebhardt et al. (2002)
Gammaproteobacteria: *Salmonella* subsp., *Pseudomonas fluorescens*[b]	*Pachyiulus flavipes*	Midgut fluid	Byzov (2003)
Betaproteobacteria: *Alcaligenes xylosooxidans, Burkholderia cepacia*[b]			

[a] Methanogenic bacteria were detected microscopically by their characteristic blue autofluorescence (Hackstein and Stumm 1994)
[b] Strains identified by BIOLOG

Table 4.2. Taxonomic composition of yeasts and mycelial fungi isolated from the millipede guts

Genus/species	Host	Source of isolation	Reference
Ascomycetous: *Debaryomyces hansenii, Torulaspora delbrueckii, Zygowilliopsis californicus* (=*Williopsis californica*), *Galactomyces geotrichum*	*Pachyiulus flavipes*	Midgut (intestinal walls + gut contents)	Byzov et al. (1993a)
Ascomycetous: *Debaryomyces hansenii, Torulaspora delbrueckii, Zygowilliopsis californicus* (=*Williopsis californica*), *Pichia membranaefaciens, Zygosaccharomyces rouxii, Saccharomyces paradoxus, Saccharomyces transvaalensis, Arthroascus schoenii, Kluyveromyces thermotolerans, Citeromyces matritensis, Pichia anomala, Galactomyces geotrichum, Candida valida, Candida ingens, Candida norvegica, Candida friedrichii, Kloeckera apis* **Basidiomycetous:** *Cryptococcus albidus, Trichosporon pullulans, Ttichosporon beigelii, Trichosporon cutaneum, Aureobasidium pullulans, Rhodotorula rubra*	*Pachyiulus flavipes*	Hindgut (intestinal walls+gut contents) and fresh excrements	Byzov et al. (1993a); Vu Nguyen Thanh (1993)
Ascomycetous: *Pichia guilliermondii*	*Glomeris connexa*	Hindgut (intestinal walls+gut contents)	Byzov et al. (1993a)
Trichomycetes	Diplopoda	Hindgut cuticule	Moss and Taylor (1996)
Trichomycetes: *Enterobryus* (Eccrinales)	Diplopoda	Hindgut cuticule	Alencar et al. (2003)
Unidentified yeast-like fungi	*Ommatoiulus sabulosus*	Gut content	Jarosz and Kania (2000)

4.5.1 Bacteria

Most studies of the bacterial diversity in the intestinal tracts of millipedes were made using the dilution plate method. In most cases, the identification of isolates was performed using phenotypic characteristics, morphological, physiological and biochemical features.

The number of aerobic, culturable bacteria was found by Ineson and Anderson (1985) to be 2.8×10^9 CFU/g dry weight in the whole gut of the diplopod *Glomeris marginata*. The ratio of viable bacterial counts to direct counts was lower in litter than in the gut and faeces of this diplopod, suggesting that the gut environment enhanced bacterial growth and viability (Anderson and Bignell 1980). In *Pachyiulus flavipes*, the total CFU counts of bacteria were found to be $1.0–7.8 \times 10^8$ CFU/g dry weight (Byzov et al. 1993c; Tret'yakova et al. 1996) with increasing counts from the foregut to the midgut, and to the hindgut, 0.15, 1.94 and 2.7×10^8, respectively (Tret'yakova et al. 1996). Lower bacterial counts have been recorded in the guts of the diplopods *Chromatoiulus rossicus* and *Glomeris connexa,* from 1.0×10^6 to 2.1×10^7 and from 8.0×10^6 to 2.7×10^7 CFU/g dry weight, respectively. The numbers of bacteria were quite similar in the midgut and hindgut. The numbers of bacteria isolated from the peritrophic membrane of *G. connexa* were similar to those for gut tissue, about 10^7 CFU/g dry membrane (Byzov et al. 1996a).

Most of bacterial strains isolated from the millipede guts and the gut contents belong to the gamma subclass of Proteobacteria and the phylum Actinobacteria, class Actinobacteria. The dominant bacteria found by many authors inhabiting the guts belong to facultative anaerobic bacteria of the family Enterobacteriaceae, genera *Klebsiella, Enterobacter, Plesiomonas, Salmonella, Erwinia, Escherichia,* and of the family Vibrionaceae, genus *Vibrio* (Table 4.1). It has been shown these two families of bacteria predominate on the washed intestinal walls of *Glomeris connexa, Leptoiulus polonicus* and *Pachyiulus flavipes*. They occupied all the gut parts but were most numerous in the hindgut. Their numbers ranged from 1.0×10^6 to 2.7×10^7 CFU/g dry gut weight; they represented 50–80% of all isolates. These bacteria were consistently isolated during several months of laboratory rearing of the animals; they were not isolated from the food (leaf litter); their populations remained relatively stable in the starving animal. These can be indirect evidence for their intestinal origin (Byzov et al. 1996a; Tret'yakova et al. 1996).

The second numerous group of bacteria, inhabiting the guts, is Actinobacteria. Among them were found representatives of the families Promicromonosporaceae, Cellulomonadaceae and Streptomycetaceae with *Promi-*

cromonoposra-Oerskovia group – nocardioform actinomycetes and *Streptomyces* predominated (Table 4.1).

Szabó and his group first isolated nocardioform actinomycetes from millipede guts (Dzingov et al. 1982; Szabó et al. 1983). The first species, the strain of which was isolated from the gut and faeces of *Chromatoiulus projectus* and formed large populations in the hindgut,was described as *Promicromonospora enterophila* (Jager et al. 1983). It was considered a true intestinal associate because it disappeared quickly from fresh excrement as it aged (Márialigeti et al. 1985). *Promicromonospora*-type bacteria were also found in the gut, the gut contents and fresh faeces of *Chromatoiulus rossicus, Leptoiulus proximus, Cylindroiulus luridus, C. boleti, Unciger foetidus* and *Pachyiulus flavipes* (Table 4.1). Another nocardioform monospore actinomycete has been isolated from the gut and faeces of *Glomeris hexasticha.* It was supposed that it represented an intermediate taxon between the genera *Oerskovia* and *Promicromonospora.* This indigenous intestinal microbe was completely absent from, or occurred sporadically, in the soil or litter of its host animal's feeding habitat. They do not multiply in soils in the presence of complex natural microflora. These data corroborated the conclusion of Anderson and Bignell (1980) that the specific gut symbionts do not proliferate in millipede faeces. This can be considered as indirect evidence of symbiotic origin of the gut actinomycetes. Direct evidence of the intestinal origin of nocardioforms can be drawn from the finding that these actinomycetes were isolated from the washed intestinal walls (Byzov et al. 1993c; Tret'yakova et al. 1996). It was concluded that nocardioform gut populations of different millipede species might belong not only to different species but also to various genera of actinomycetes (Chu et al. 1987).

Various streptomycete species have been isolated from both millipede gut walls and gut contents (Table 4.1). They were also found on the peritrophic membrane in the midgut (Byzov et al. 1993c; Nguyen Duc et al. 1996; Tret'yakova et al. 1996). The presence of actinomycete mycelium (less than 1 μm in diameter) on the intestinal wall in the hindgut of *Pachyiulus flavipes*, revealed by scanning electron microscopy (Byzov et al. 1993c), could be considered evidence that this actinomycetes belong to true intestinal microbiota. Numbers of streptomycetes reached 10^5 CFU/g gut tissue. Different species of *Micromonospora, Actinomadura* and *Streptosporangium* were also isolated, of which the numbers were less than 10^4 CFU/g. However, it was difficult to evaluate the real density of actinomycetes because of the limitation to count mycelial spore forming organisms by the dilution plate method (Byzov et al. 1993c; Nguyen Duc TL et al. 1996).

A strain of *Bacillus brevis,* which produced antifungal metabolites, was isolated from the millipede *Glomeris* sp. (Gebhard et al. 2002), but there was weak evidence than this was an endosymbiont.

Free methanogenic bacteria and ciliates (*Nyctotherus* type) with intracellular endosymbiotic methanogens have been detected microscopically by their characteristic autofluorescence in the hindguts of the tropical diplopods *Chicobolus* sp., *Orthoporus* sp., *Rhapidostreptus virgator* and two unidentified species. The ciliates carried > 4000 methanogens per ciliate cell. Methanogens were also found in their cysts. Unlike other arthropods, in which the methanogens were found in the hindgut pouch, the millipedes do not have such a pouch. The estimation of global methane production, based on laboratory measurements, shows that several species of millipedes (with and without intestinal protists) contribute a significant quantity of methane compared to that emitted by other arthropods. However, some species of diplopods did not emit methane, probably due to a secondary loss of the methanogens (Hackstein and Stumm 1994).

Among the bacterial isolates those with cellulase activity were not found with the exception of the study by Taylor (1982). Therefore, it is unlikely that millipedes possess cellulolytic symbionts as the termites and other insects.

4.5.2 Fungi

Trichomycetes (Zygomycota) are obligate symbionts that live in the digestive tract of various arthropods, including Diplopoda (White et al. 2000). The relationships of Trichomycetes to their hosts is generally commensalistic or pathogenesis, and, in some cases, mutualistic, depending on developmental and environmental conditions (Lichtwardt 1996). The Hindguts of many species of millipedes (Diplopoda) throughout the world are hosts to *Enterobryus* (Eccrinales) (Lichtwardt 1996), the first genus of Trichomycetes to be named. Alencar et al. (2003) reported one species of *Enterobryus* that is probably new, which was found in a small spirobolid millipede. The feature that makes this eccrinid unusual is the presence of extremely long holdfasts in mature thalli revealed by phase contrast microscopy of the hindgut. Holdfasts attach thalli to the hindgut cuticle, and are extruded through pores in the wall at the base of the thalli.

Most of yeast strains isolated from the millipede guts and the gut contents are ascomycetous. In *Pachyiulus flavipes*, the predominating species were *Debaryomyces hansenii, Torulaspora delbrueckii, Zygowilliopsis californicus* (=*Williopsis californica*) and *Pichia membranaefaciens*. Byzov et al. (1993a) determined counts up to 10^5 CFU/g in the hindgut, and less than 10^3 CFU/g in the midgut (Table 4.2). These yeasts proved to be obligate gut associates of millipedes. The yeasts dominated in the gut were not found in the animal food but survived gut passage. They are consistently present

in the gut of the diplopods reared under different external conditions such as long-term starvation, feeding of sterile substrates and artificial "wintering" of animals maintaining their composition and numbers at a relatively steady state. The fact that regardless of food quality and feeding regimens the composition of gut yeasts remained constant can be considered as indirect evidence of a very close association with the host organism (Byzov et al. 1993a,b). The gut yeasts are characterized by a fermentative metabolism and have a very narrow assimilation spectrum (Vu Nguyen Thanh 1993). These facts correspond to facultative anaerobic conditions (Bignell 1984) and high concentrations of glucose (Byzov 2003) in the hindgut of millipedes. Jarosz and Kania (2000) isolated yeast-like fungi and moulds from the gut content of *Ommatoiulus sabulosus* occurred at low population densities but species were not identified.

4.6 Functions of the Intestinal Microbiota

Functional importance of gut microorganisms for soil millipedes has not been generally investigated. There is indirect evidence that microbial associates participate in the digestion and can provide the animals with food. They can act as pathogenic agents or cause the colonization resistance. There is an indication that gut microbes produce methane (Table 4.3).

4.6.1 Digestive Functions of Gut Microorganisms

It was found that the efficiency of ^{14}C-cellulose breakdown and the assimilation by the desert millipedes *Orthoporus ornatus* and *Comanchelus* sp. were reduced if antibiotics are incorporated into the food. It was concluded that the millipede-bacterium association was mutualistic that enables millipedes to utilize otherwise unavailable plant polymers (Taylor 1982).

An in vitro study has demonstrated that gut actinobacteria isolated from the hindgut of the millipede *Pachyiulus flavipes* (coryneforms and streptomycetes) may kill and digest living yeast cells of *Debaryomyces hansenii* – one of the predominating gut species. It appears that this kind of symbiotic digestion may be important for the millipede (Byzov et al. 1993b). Similar antagonistic role of actinomycetes in the digestion of microorganisms was shown for soil feeding termites (Bignell et al. 1983).

In the hindgut of *P. flavipes*, unicellular bacteria and actinomycetes hyphae were found on the remnants of the peritrophic membrane, indicating that decomposition of this chitinous material takes place (Byzov et al. 1993b,

Table 4.3. Microbial associates and their possible functions in the digestive tract of millipedes

Microorganisms/ microhabitat	Function	Host	Reference
Unidentified bacteria/midgut, hindgut	**Digestion:** cellulose and hemicellulose, and pectin degradation	*Orthoporus ornatus, Comanchelus sp.*	Taylor (1982)
Klebsiella pneumonia, unidentified bacteria/faeces	**Digestion:** acetate as sole C-source	*Glomeris marginata*	Ineson and Anderson (1985)
Actinomycetes/hindgut	**Digestion:** destruction of peritrophic membrane	*Pachyiulus flavipes*	Byzov et al. (1993c)
Actinomycetes/hindgut	**Digestion:** yeast-lysing activity	*Pachyiulus flavipes*	Byzov et al. (1993b)
Oerskovia-Promicromonospora-Nocardioforms (OPNs)/hindgut	**Food for the host:** digestion of the gut OPNs	*Chromatoiulus projectus*	Szabó et al. (1985)
Yeasts of DTZ-group/hindgut	**Food for the host:** digestion of symbiotic yeasts	*Pachyiulus flavipes*	Byzov et al. (1993b)
Bacillus cereus/hindgut	**Antagonistic interaction:** heightened antibiotic activity against *Promicromonospora enterophila*	*Cylindroiulus boleti*	Contreras (1985)
Streptomyces chrysomalus, S. candidus/hindgut; *Streptomyces fluorescens, S. xanthoacidicus*/peritrophic membrane	**Resistance to colonization:** heightened Antibiotic activity against Gram-positive bacteria and yeasts	*Pachyiulus flavipes*	Nguyen Duc TL et al. (1996)
Enterobacter agglomerans, Escherichia coli, Klebsiella sp./gut content	**Resistance to colonization:** rapid colonization and overgrowth	*Ommatoiulus sabulosus*	Jarosz and Kania (2000)
Bacillus brevis/hindgut	**Resistance to colonization:** production of antifungal antibiotic bacillomycin D	*Glomeris* sp.	Gebhard et al. (2002)
Candida guilliermondii/hindgut	**Pathogenic agent:** overgrowth the whole gut	*Glomeris connexa*	Byzov et al. (1993b)

Table 4.3. (continued)

Microorganisms/ microhabitat	Function	Host	Reference
Unidentified yeasts/whole gut	**Pathogenic agent:** overgrowth the whole gut	*Leptoiulus polonicus, Megaphyllum projectum*	Byzov et al. (1993b)
Klebsiella pneumonia, unidentified bacteria/faeces	**Detoxification of nitrogenous wastes:** urease and uricase activities in vitro	*Glomeris marginata*	Ineson and Anderson (1985)
Yeasts: *Debaryomyces hansenii, Zygowilliopsis californicus, Torulaspora delbrueckii* (DTZ group), *Candida guilliermondii, Kloekera apis, Trichosporon cutaneum, T. pullulans, Geothrichum candidum*/midgut, hindgut	**Detoxification of nitrogenous wastes:** utilization of the waste end product uric acid as the sole N-source	*Pachyiulus flavipes*	Byzov et al. (1993b)
Methanogens/hindgut	**CH_4 and H_2** production	*Chicobolus* sp.	Hackstein and Stumm (1994)
Free methanogenic bacteria/hindgut; ciliates with intracellular methanogens/hindgut		*Orthoporus* sp., *Rhapidostreptus virgator*; two unidentified species	
Not determined		*Pycnotropis acuticollis*	
Free methanogenic bacteria/hindgut		two unidentified species	
No methanogens	**H_2**production	*Glomeris* sp.	

1996a). This may imply re-utilization of nitrogen-containing substrates and nitrogen balance in millipedes.

Another possible mechanism of nitrogen balance could be the utilization of the end products urea and uric acid by gut microorganisms. It has been demonstrated that bacteria isolated from the faeces of *Glomeris marginata* possessed urease and uricase that was less frequent for litter isolates (Ineson and Anderson 1985). The yeasts isolated from *P. flavipes* were able to grow on uric acid as a sole source of nitrogen (Byzov et al. 1993b). The urea and the uric acid are toxic and their detoxification must be important for the animals.

4.6.2 Intestinal Microbiota as a Food for Millipedes

Gut microbes can provide food for the host. It was hypothesized by Szabó et al. (1985) that the millipede *Chromatoiulus projectus* could utilize the cell materials of *Oerskovia-Promicromonospora-Nocardioforms*. It was found that the yeasts *Debaryomyces hansenii, Torulaspora delbrueckii, Zygowilliopsis californicus* that live in the hindgut of the millipede *Pachyiulus flavipes* did not occur in its food, but could be isolated at lower densities from the midgut. On the other hand, these microorganisms were sensitive to the digestive fluid of the host. This may indicate the yeast cells are transported to the digestive region of the gut by antiperistaltic movement and lysed there. Such an endogenous feeding might be important in starving animals (Byzov et al. 1993b).

It has been calculated that gut microorganisms might provide the millipedes with essential amino acids whose concentrations are low in plant litter (Pokarzhevskii et al. 1984).

Gut microorganisms may apparently directly supply the host with nutritive substrates. The hypothesis follows from the fact that ^{14}C-labelled yeasts *Debaryomyces hansenii* lost up to 80% of ^{14}C within 30 min incubation in the midgut fluid without loosing their viability (Byzov 2003).

These are mutualistic interactions when the host organism provides microorganisms with favourable place of residence, namely the hindgut. The hindgut of millipedes is a natural fermenter. The Malphigian tubules excrete mineral compounds, urea and uric acid that are transported into the hindgut space (Bignell 1984). Carbohydrates are also transported there as part of the food. Thus, there are all the essential nutrients in the hindgut to promote microbial growth.

4.6.3 Resistance to Colonization

One of the important functions of the intestinal microbiota is to protect the intestine from colonization by external microorganisms. Nguyen Duc TL et al. (1996) have found that actinomycetes isolated from the hindgut of *Pachyiulus flavipes* showed in vitro considerable higher antibiotic activity against bacteria as compared to litter isolates. Bacteria, isolated from the animal guts, were more sensitive than those isolated from litter. Moreover, Gram-positive bacteria were more sensitive than Gram-negative. It was concluded that the heightened antagonistic activity makes the actinomycetes more competitive with other Gram-positive bacteria. At the same time, they do not compete with Gram-negative bacteria that predominate in

the community. Similar results were obtained by Szabó (1974) who found that actinomycetes isolated from larval Bibionidae (Diptera) were more competitive with each other than with other gut bacteria.

The bacterium *Bacillus brevis* has been isolated from the hindgut of the millipede *Glomeris* sp.The culture filtrate extract of the bacterium exhibited strong antifungal activity (against standard strains for testing *Saccharomyces cerevisiae, Botrytis cinerea* and *Cladosporium cucumerinum*). The activity was explained by the presence of a member of the lipopeptide antibiotic of the iturin group. It was identified as bacillomycin D. It was also shown that this bacterial strain produced the lipopeptide antibiotics fengycin and surfactin (Gebhardt et al. 2002). These phenomena, however, were not demonstrated in vivo.

Jarosz and Kania (2000) have pointed out that the specific composition of bacterial flora in the millipede intestines cannot be explained simply by antibiotic inhibition of contaminating microflora. They have shown that the predominant types of gut enterobacteria of *Ommatoiulus sabulosus* were unable to produce in vitro any bacteriolytic activity of lysozyme-like enzymes, bacteriocins or other antimicrobial molecules. The lack of microbial contaminators could rather result from the unfavourable biochemical niche in the midgut enzymes and little or no competition for the enteric bacteria group predominant in the millipede alimentary tract. The rapid colonization and overgrowth of the intestines eliminate bacteria, yeasts and moulds ingested with food. Killing action of the midgut fluid against litter microorganisms was found earlier (Byzov et al. 1993b, 1998a)

4.6.4 Intestinal Microbiota as a Pathogenic Agent

These balanced interactions between the host and its intestinal microbiota can change into a pathogenic state if the natural protective ability of the midgut is disturbed. It can happen if unnaturally high amounts of gut microorganisms are introduced, following removal of natural microbiota. In the feeding experiment it was shown that the yeasts *Candida guilliermondii*, which normally inhabit the hindgut of diplopods *Glomeris connexa*, can cause the disease when their natural density is increased 100 times. To be killed by yeasts, a total colonization of the midgut epithelium is necessary. This can happen naturally during the long-term rearing of diplopods under laboratory conditions (Byzov et al. 1993b). The mechanism of the effect is unknown but it could be metabolic (yeast toxins, heightened CO_2 production due to yeast growth) and trophic (competition for nutrition, vitamins).

4.7 Digestion of Microorganisms by Millipedes

It is well known that soil millipedes not only regulate the numbers of microorganisms but they also modify microbial communities by eliminating some and enabling the growth of others (Anderson and Bignell 1980; Ineson and Anderson 1985, among others). The mechanism of such a selection is not fully understood; however, it is believed that the first barrier for those microorganisms that enter the gut is the killing activity of the midgut environment.

4.7.1 Killing Activity of the Midgut Fluid

The digestive midgut fluid of the millipede *Pachyiulus flavipes* has been shown to exhibit a selective biocidal killing activity against a variety of microorganisms: bacteria, actinomycetes, yeasts and filamentous fungi (Thanh et al. 1994). Assays using a range of microorganisms found their sensitivities to the fluid to be species and strain dependent. The effects ranged from those sensitive to resistant ones with the isolates of the millipede guts being the most resistant (Thanh et al. 1994; Byzov and Rabinovich 1997; Byzov et al. 1998a,b). The digestive fluid has also been found to kill some soil invertebrates, e.g. nematodes, enchytraeids, ants (B. Byzov, unpubl. data). A similar type of activity has been found in the digestive fluid of two other millipedes, *Rossiulus kessleri* and *Megaphyllum rossicum* (Byzov 2003). The killing activity was not found in the hindgut water extract (Byzov et al. 1996b).

4.7.2 Killing Effect

Those microorganisms that were found to be sensitive to the digestion were killed after only 1–2 min of incubation in the fluid. The longer incubation of the killed cells resulted in a subsequent total destruction of the cells. The effects were similar to both sterile filtered digestive fluid and with fluid obtained from the animals fed on sterile cellulose or cellulose enriched with streptomycin, thus pointing to an animal rather than a bacterial origin of the effect. The rapid death of the sensitive yeast *Saccharomyces cerevisiae* was accompanied by an immediate destruction of vacuoles, nuclei and membranes; however, the cell walls of the majority of the cells remained intact within 1–3 h of incubation (Byzov et al. 1998b).

4.7.3 Properties of the Killing Compound(s)

Byzov and Rabinovich (1997) separated the biocidal compounds from the native digestive fluid by a combination of chloroform-methanol extraction and LH-20 Sephadex chromatography. The concentration of the biocidal compounds was about 15 mg/ml. The biocidal activity was found to be associated with the non-protein fraction. The biocidal compounds can be dissolved in water, 50%-methanol and chloroform; they are thermostable (98 °C, 10 min), and act effectively down to a sixfold dilution of the native fluid.

Some purified biocidal fractions were water surface active. A decrease of surface tension of water from 72 to 42 mN/m caused by 1:100 or less diluted fraction is characteristic of a moderate surfactant. Elucidation of molecular structure of the killing compounds in the millipede *Pachyiulus flavipes* by mass spectrometry has revealed the presence of saturated fatty acids with different chain lengths carrying a hydrophilic group in the omega position. The preliminary structural and computer analysis showed that these compounds are not yet registered in the American Chemical Society Database, which documents all known chemical structures (Golyshin et al., unpubl. data).

4.7.4 Induced Autolysis

The killing compounds found in the digestive fluid of soil millipedes seem to play an important role in the digestive process in the animals. The microbiolytic activity of the midgut fluid most likely relates to its protein fraction. However, neither the protein nor the biocidal fractions caused the destruction of the cells, when applied separately. Complete destruction of the cells was only observed when a mixture of the two fractions or the native fluid was applied. The digestion process apparently involves two steps: microbial cells are initially killed by the non-protein substances and then hydrolysed by both midgut hydrolytic enzymes and autolytic enzymes of the microbial prey. Induced autolysis is suggested to play an important role in the digestion of microorganisms by the millipede (Byzov et al. 1998a,b).

4.7.5 Assimilation of Microorganisms

Wolters and Ekschmitt (1997) observed that the assimilation efficiency of microorganisms by millipedes is high and exceeds that of plant material

(which is generally < 20%). In feeding experiments with ^{14}C-labelled microbes, it was shown that in *Glomeris marginata* the assimilation efficiency reached 96.9% for *Erwinia herbicola,* 93.2% for *Pseudomonas syringae*, 82,6% for *Bacillus subtilis*, 72.2% for *Escherichia coli* and 73.5% for *Mucor hiemalis* (Bignell 1989). In *Pachyiulus flavipes,* it was 73% for *Rhodotorula graminis,* 80% for *Trichosporon pullulans,* 82% for *Debaryomyces hansenii*, 60% for hyphae of *Streptomyces pseudogriseolus* and 40% for *S. californicus* (Byzov et al. 1998).

4.8 Conclusions

Since the pioneer works in the 1980s, the intestinal microorganisms of soil millipedes have been mostly studied by routine isolation techniques and microscopy. Nevertheless, many interesting discoveries have been made. Almost 30 species of diplopods have been studied, most of which belonged to the family Julidae. A diverse microbiota has been found in the gut comprising facultative anaerobic enterobacteria, actinomycetes, nocardioforms, yeasts and fungi. It could be concluded that the intestinal microbiota of different diplopods has common features on generic and higher levels, but there is a high microbial diversity at the species level. A number of studies are indicative of intimate associations between microorganisms and the millipede gut. The most important are those that demonstrate possible functions of gut microbiota. Among them digestive activity, maintaining microbial community at steady state, in vivo production of methane. The discovery of killing mechanism can partially explain how diplopod digestive system is organized. It is most likely that these animals do not possess symbiotic microorganisms, capable of digesting lignocelluloses, as is the case in wood-feeding termites and other insects. It is believed that they feed on microorganisms and use microbial enzymes to digest recalcitrant molecules. Such a mechanism was suggested for isopods and soil-feeding termites and probably exists in other soil invertebrates, e.g. earthworms. Gut microbes are also important as the source of essential amino acids.

Fortunately, there are many starting points to continue more either detailed or extensive researches in the field of microbial-millipede interactions. Modern methods of isolation and identification of microorganisms and in situ studies open promising perspectives for enthusiasts to discover new symbiotic microorganisms and describe their ecological functions.

Acknowledgements. The author wishes to thank Professor Jonathan M. Anderson and Dr. Peter N. Golyshin for their useful comments and editing

the manuscript. This work was partially supported by the grant from the Russian Foundation for Basic Researches (Projects no. 02-04-49049).

References

Alencar YB, Ríos-Velásquez CM, Lichtwardt RW, Hamada N (2003) Trichomycetes (Zygomycota) in the digestive tract of arthropods in Amazonas, Brazil. Mem Inst Oswaldo Cruz, Rio de Janeiro, 98, in press

Anderson JM, Bignell DE (1980) Bacteria in the food, gut contents and faeces of the litter feeding millipede *Glomeris marginata*. Soil Biol Biochem 12:251–254

Anderson JM, Bignell DE (1982) Assimilation of ^{14}C-labelled leaf fibre by the millipede *Glomeris marginata* (Diplopoda, Glomeridae). Pedobiologia 23:120–125

Anderson JM, Ineson P (1984) Interactions between soil arthropods and microbial populations in carbon, nitrogen and mineral nutrient fluxes from decomposing leaf litter. In: Lee JA,

Baleux B, Vivares ChP (1974) Etude préliminaire de la florebactérienne intestinale de *Schizophyllum sabulosum* var. *rubripes* Lat. (Myriapoda, Diplopoda). Bull Soc Zool France 99:771–779

Beck L, Friebe B (1981) Verwertung von Kohlenhydraten bei *Onuscus asellus* (Isopoda) und *Polydesmus angustus* (Diplopoda). Pedobiologia 21:19–29

Bignell DE (1984) The arthropod gut as an environment for microorganisms. In: Anderson JM, Rayner ADM, Walton DWH (eds) Invertebrate-microbial interactions. Cambridge University Press, Cambridge-London-New York-New Rochelle-Melbourne-Sydney, pp 206–227

Bignell DE (1989) Relative assimilation of ^{14}C-labelled microbial tissue and ^{14}C-plant fibers ingested with leaf litter by the millipede *Glomeris marginata* under experimental conditions. Soil Biol Biochem 21:819–827

Bignell DE, Oskarsson H, Anderson JM, Ineson P, Wood PG (1983) Structure, microbial associations and function of the so-called 'mixed segment' of the gut in two soil-feeding termites, *Procubitermes aburiensis* Sjostedt and *Cubitermes severus* Silvestri (Termitidae, Termitinae). J Zool 201:445–480

Byzov BA (2003) Zoo-microbial interactions in soil. Thesis of Dissertation Doctor of Microbiology and Soil Science, MSU (in Russian)

Byzov BA, Rabinovich YaM (1997) The killer activities of the digestive fluid of the millipede *Pachyiulus flavipes*. Appl Biochem Microbial 33:507–509

Byzov BA, Vu Nguyen Thanh, Bab'eva IP (1993a) Yeasts associated with soil invertebrates. Biol Fertil Soils 16:183–187

Byzov BA, Vu Nguyen Thanh Bab'eva IP (1993b) Interrelationships between yeasts and soil diplopods. Soil Biol Biochem 25:1119–1126

Byzov BA, Zenova GM, Babkina NI, Dobrovolskaya TG, Tretjakova EB, Zvyagintsev DG (1993c) Actinomycetes in the food, gut and faeces of soil millipede, *Pachyiulus flavipes* C.L. Koch. Microbiology 62: 916–927 (in Russian)

Byzov BA, Chernjakovskaya TF, Zenova GM, Dobrovolskaya TG (1996a) Bacterial communities associated with soil diplopods. Pedobiologia, 40:67–79

Byzov BA, Claus H, Tretyakova EB, Zvyagintsev DG, Filip Z (1996b) Effects of soil invertebrates on the survival of some genetically engineered bacteria in leaf litter and soil. Biol Fertil Soils 23:221–228

Byzov BA, Kurakov AV, Tretyakova EB, Vu Nguyen Thanh, Nguyen Duc To Luu, Rabinovich YaM (1998a) Principles of the digestion of microorganisms in the gut of soil millipedes: specificity and possible mechanisms. Appl Soil Ecol 9:145–151

Byzov BA, Vu Nguyen Thanh, Bab'eva IP, Tretyakova EB, Dyvak IA, Rabinovich YaM (1998b) Killing and hydrolytic activities of the gut fluid of the millipede *Pachyiulus flavipes* C.L. Koch on yeast cells. Soil Biol Biochem 30:1137–1145

Cazemier AE, Hackstein JHP, Op den Camp HJM, Rozenberg J, van der Drift C (1997) Bacteria in the intestinal tract of different species of arthropods. Microb Ecol 33:189–197.

Chu TL, Szabó IM, Szabó I (1987) Nocardioform gut actinomycetes of *Glomeris hexasticha* Brandt (Diplopoda). Biol Fertil Soils 3:113–116

Contreras E (1985) The composition of gut bacterial communities of the millipede species *Chromatoiulus projectus* Verh. and *Cylindroiulus boleti* Koch. Thesis (Cand Sci) Acad Sci Hung, Budapest

Crawford CS, Minion GP, Bayers MD (1983) Intima morphology, bacterial morphotypes, and effects of annual molt on microflora in the hindgut of the desert millipede *Orthoporus ornatus* (Girard) (Diplopoda: Spirostreptidae). Int J Insect Morphol Embryol 12:301–312

Dzingov A, Jager K, Contreras E, Marialigeti K, Szabo IM (1982) Studies on the microflora of millipedes (Diplopoda). I. A comparison of actinomycetes isolated from surface structures of the exoskeleton and the digestive tract. Pedobiologia 24:1–7

Gebhardt KJ, Schimana J, Müller J, Fiedler H-P, Kallenborn HG, Holzenkämpfer M, Krastel P, Zeeck A, Vater J, Hötzel A, Schmid DG, Rheinheimer J, Dettner K (2002) Screening for biologically active metabolites with endosymbiotic bacilli isolated from arthropods. FEMS Microbiol Lett 217:199–205

Guzev VS, Zvyagintsev DG (2003) The biometric analysis of bacterial cells in soil. Microbiology 72:187–192

Hackstein JHP, Stumm CK (1994) Methane production in terrestrial arthropods. Proc Natl Acad Sci USA 91:5441–5445

Handlon RDG (1981) Some factors influencing microbial growth on soil animal faeces. 2 Bacterial and fungal growth on soil animal faeces. Pedobiologia 21:264–270

Hopkin SP, Read HJ (1992) The biology of millepedes, Oxford-New York-Tokyo, Oxford University Press

Ineson P, Anderson JM (1985) Aerobically isolated bacteria associated with the gut and faeces of the litter feeding macroarthropods *Oniscus assellus* and *Glomeris marginata*. Soil Biol Biochem 17: 843–849

Jager K, Marialigeti K, Hauck M, Barabás G (1983) *Promicromonospora enterophila* sp. nov., a new species of monospore actinomycetes. Int Journ System Bacteriol 33:525–531

Jarosz J, Kania G (2000) The question of whether gut microflora of the millipede *Ommatoiulus sabulosus* could function as a threshold to food infections. Pedobiologia 44: 705–708

Kaplan DL, Hartenstein R (1978) Studies on monoxygenases and dioxygenases in soil macroinvertebrates and bacterial isolates from the gut of the terrestrial isopod *Oniscus asellus* L. Comp Biochem Physiol B, 60:47–50

Lichtwardt RW (1996) Trichomycetes and the arthropod gut. In: Howard D and Miller D (eds) The Mycota, animal and human relations, Springer-Verlag, New-York, pp 315–330

Marcuzzi G, Turchetto LH (1975) Observation on the digestive enzymes of some litter feeding animals. In: Progress in Soil Zoology, Prague, pp 593–599

Marcuzzi G, Turchetto-Lafisca ML (1977) On lipases in litter feeding invertebrates. Pedobiologia 17:135–144

Marialigeti K, Contreras E, Barabás Gy, Heydrich M, Szabó IM (1985) True intestinal actinomycetes of Millipedes (Diplopoda). J Invertebr Pathol 45:120–121
Martin JS, Kirkham JB (1989) Dynamic role of microvilli in peritrophic membrane formation. Tissue Cell 21:627–638
Moss ST, Taylor JE (1996) Mycobionts in the guts of millipedes – the Eccrinales. Mycologist 10:121–124
Neuhauser E, Hartenstein R (1976) On the presence of O-dimethylase activity in invertebrates. Comp Biochem Physiol C 53:37–39
Neuchauser E, Hartenstein E, Connors WJ (1978) Soil invertebrates and the degradation of vanillin, cinnamic acid, and lignins. Soil Biol Biochem 10:431–435
Nguyen Duc TL, Byzov BA, Zenova GM, Zvyagintsev DG (1996) Antagonistic properties of actinomycetes associated with intestinal tract of soil invertebrates. Vestnik Mosk. Un-ta 3:70–77 (in Russian)
Nielsen C.O. Carbohydrates in soil and litter invertebrates. Oikos, 1962, V. 13, pp. 200–215
Nunez FS, Crawford CS (1976) Digestive enzymes of the desert millipede *Orthoporus ornatus* (Girard) (Diplopoda, Spirostreptida). Comp Biochem Physiol 55:141–145
Pokarzhevskii AD, Sikora I, Gordienko SA (1984) Amino-acid resources in the food of saprophages. Dokl Acad Nauk SSSR 277: 253–256
Polyanskaya LM, Babkina NI, Zenova GM, Zvyagintsev DG (1996) Fate of actinomycetes in the intestinal tract of soil invertebrates fed on streptomycete spores. Microbiology 65:493–498
Striganova BR (1970) Cellulose decomposition in the gut of *Pachyiulus foetidissimus* (Mur.) (Julidae, Diplopoda). Dokl Acad Nauk SSSR 190:703–705
Szabó IM (1974) Microbial communities in a Forest-Rendzina ecosystem. The Pattern of Microbial Communities, Acad. Press, Budapest
Szabó IM, Chu TL, Contreras E, Heydrich M, Jáger K, Marialigeti K, Pobozsny M, Ravasz K, Zicsi A (1987) On the problems of intestinal bacteriology of forest-litter consuming invertebrates. In: Striganova BR (ed) Soil fauna and soil fertility. Proc 9th Int Coll Soil Zool, Moscow, Nauka, 58–63
Szabó IM, Jager K, Contreras E, Marialigeti K, Dzingov A, Barabás Gy, Pobozsny M (1983) Composition and properties of the external and internal microflora of millipedes. In: Lebrun Ph, André HM, De Medts A, Grégoire-Wibo, Wauthy G (eds) New Trends in Soil Biology, Imprimeur Dieu-Brichart, Ottignies-Louvain-la-Neuve, pp 197–205
Szabó IM., Marialigeti K, Chu T, Jáger K, Szabó I, Contreras E, Ravasz K, Heydrich M, Palik E (1985) On the ecology of nocardioform intestinal actinomycetes of millipedes (Diplopoda). In: Szabó G, Biró S, Goodfellow M (eds) Proc 6th Int Symp Actinom Biol, pp 701–705
Taylor E (1982) Role of aerobic microbial populations in cellulose digestion by desert millipedes. Appl Environ Microbiol 44:281–291
Thanh VN, Byzov BA, Babjeva IP (1994) The sensitiveness of yeasts at the digestive fluid in the gut of soil diplopods, *Pachyiulus flavipes* C.L. Koch. Microbiology 63:715–720 (in Russian)
Tret'yakova EB, Dobrovol'skaya TG, Byzov BA, Zvyagintsev DG (1996) Bacterial communities associated with soil invertebrates. Microbiology 65:91–97
Vu Nguyen Thanh (1993) Fate of yeasts in associations with soil invertebrates. Thesis of Dissertation Candidate of Science (Microbiology), Moscow, MSU, 25 pp
White MM, Cafaro MJ, Lichtward RW (2000) Arthropod gut fungi from Puerto Rico and summary of tropical Trichomycetes worldwide. Caribbean J Sci 36:210–220
Wolters V, Ekschmitt K (1997) Gastropods, isopods, diplopods and chilopods – neglected groups of the decomposer food web. In: Benckiser G (ed) Fauna in soil ecosystems. New York, Marcel Dekker, Inc., pp 265–306