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ECOLOGY

Microbial Population of the Digestive Tract of Click Beetle Larvae (Elateridae, Coleoptera)

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Abstract—The composition and functional structure of the intestinal microflora of three wireworm species (Agriotes obscurus (L.), Selatosomus aeneus (L.), and Ampedus pomorum (Herbst)) with different dietary regimes were studied. The total abundance of the microorganisms was evaluated by fluorescent microscopy, the group composition was assessed by inoculation on a solid glucose-peptone-yeast medium, and the functional diversity was estimated by multisubstrate testing. It was noted that, in the intestine of the larvae, the total number of microorganisms was lower by 1–2 orders of magnitude than in the soil and decaying wood. It was found that the composition of the intestinal microbial communities of wireworms was radically different from that of the substrate: the Bray-Curtis coefficient did not exceed 0.25. It was found that native forms accounted for more than half of the total number of saprotrophic bacteria: in the larvae, Gram-positive cocci, enterobacteria, Vibrionaceae, Acinetobacter, and some genera of coryneform bacteria, which were absent in the soil and wood, prevailed. The micromycetes were either absent (Agriotes) or were found in insignificant quantities (Selatosomus, Ampedus). In Selatosomus, apart from the intestinal forms, representatives of Mezorhizobium, Nocardioides, and Erwinia, occurring on plant substrates, were observed.

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INTRODUCTION

The microbial populations of the digestive tract of insects are characterized by high taxonomic and functional diversity (Broderick et al., 2004; Hongoh et al., 2006). Microorganisms can be food objects, pathogens, or antagonists of pathogens. They can be involved in digestion or provide a source of available nitrogen, vitamins, essential amino acids, and sterols (Douglas, 2009). Symbiotic relationships between insects and intestinal bacteria, which have developed in the course of evolution, are necessary for normal functioning and development of insects.

The intestinal communities of soil residents are especially interesting. Soil and decaying wood are media with a high number of microorganisms (~10^9 per 1 g of substrate), so the microbial cells will inevitably enter the digestive system of animals from the environment. Recent studies of intestinal microbial communities of soil invertebrates distinguish between native bacterial components and transit components entering into the intestine from the environment (Szabó et al., 1992; Tretyakova et al., 1996; Byzov, 2005).

The composition of the microbial community of an animal intestine is determined by several factors, including the phylogenetic relationships, mode of life of the host, diet, habitat, and age characteristics (Santo Domingo et al., 1998; Schmitt-Wagner, et al., 2003; Mohr and Tebbe, 2006; Behar et al., 2008). There is evidence that the host's diet can shift both the functional and taxonomic structure of the microbial community (Kane and Breznak, 1991; Broderick et al., 2004).

Most of the larvae of click beetles have a mixed diet with a predominance of a particular food regime. The family includes forms with different trophic relationships: from predominant predation to predominant phytophagy.

The aim of this study is to compare the microbial population of the intestine of the larvae with different food preferences (*Agriotes obscurus* (predominantly phytophagous), *Selatosomus aeneus* (phyto-saprophage requiring animal food for pupation), and *Ampedus pomorum* (predominantly predatory and necrophagous)) (Dolin, 1964), to reveal the differences determined by food specialization, and to distinguish the transit and residential components of the microbial community.

MATERIALS AND METHODS

Older larvae were collected in the summer of 2013 in the floodplain of the Karamysh River (typical steppe, Saratov oblast) from samples of soil and rotten wood. The soil larvae were collected by a hand sorting

of the soil samples, the larvae of *A. pomorum* were collected by disintegrating the rotting wood. In the laboratory, the larvae were kept individually in containers of 50 mL at constant humidity and temperature conditions, in the substrate from which they were collected.

For microbiological studies, the caudal segment of a larva's abdomen was cut off, the intestines were removed with tweezers and placed into in a 2-milliliter test tube and dissected. A mixed sample of the intestine contents of several larvae (3–5) was diluted with sterile water 1 : 100, the test tube was shaken on a VORTEX V-3 laboratory test tube shaker (Latvia), and the suspension was used for microbiological analyses. Two mixed samples were used in the analyses.

For comparison of the intestinal microbiota of wireworms, samples of the humus horizon of the floodplain soils, as well as samples of wood, inhabited by *A. pomorum* were used. To prepare a suspension, the soil and wood samples were diluted by water in a flat-bottomed test tube and sonicated using a Sonopuls HD2070 dispersant (Switzerland) for 2 min.

The total number of microorganisms was assessed by fluorescence microscopy, for which 10 μL of the soil suspension (1 : 100) was applied in triplicate repetition to a glass slide, fixed, and stained with acridine orange. The number of bacterial cells and the length of the fungal hyphae were counted under an Axioskop 2 plus fluorescent microscope (Zeiss Optics, Germany) (*Metody...*, 1991).

To determine the number of metabolically active cells of the intestinal microorganisms of *A. obscurus* and *S. aeneus*, we used the method of fluorescent in situ hybridization (FISH). The identification of bacteria by FISH was performed in the soil samples and intestinal contents of the larvae. A soil suspension (1 : 10) was sonicated on the Sonopuls HD2070 disperser, and the microbial cells were separated by centrifugation and fixed in formaldehyde (Amann et al., 1995; Sekar et al., 2003). The samples of the intestinal contents were very small, so suspensions of large dilutions were prepared. Drops of the suspension of fixed cells were applied to the slide windows for hybridization. Hybridization was performed with the appropriate fluorescently-labeled *16S* rRNA-specific oligonucleotide probes. The total number of metabolically active eubacteria, including γ-proteobacteria and actinobacteria, and archaea was determined using EUB338-mix (Daims et al., 1999), GAM42a (Manz et al., 1992), HGC69a (Roller et al., 1994), and ARCH915 (Stahl et al., 1991) probes, respectively.

These preparations were examined under a fluorescent microscope. The microscopy data were recalculated per 1 g of substrate (intestinal contents). To avoid the distorting (overstating) influence of the microsamplings of the suspension of the wireworm intestinal contents, decreasing coefficients were used when recalculating for 1 g (Polyanskaya et al., 2000). The dependence of the coefficients on the additional dilution was approximated by an exponential function with the IGOR PRO software. For the additional dilutions performed by us, the following recalculation coefficients were obtained: 9.25 for the dilution by 44.5 times (*A. obscurus*) and 6.46 for the dilution by 26.7 times (*S. aeneus*).

The taxonomic composition of the microbial community was determined by plating on a solid nutrient media in a fivefold repetition. For soil larvae, a glucose–peptone–yeast agar (GPA) and Ashby's agar with depleted nitrogen were used. The inoculations were performed only for aerobes and facultative anaerobes. As in the soil arthropods with a simple tubelike intestine, obligate anaerobes are absent. The plates were incubated in a thermostat at 28°C for seven days.

As the abundance micromycetes in the rotting wood was high, the suspensions from the intestines of the *A. pomorum* larvae and from the rotting wood were also plated on the Czapek mineral medium for fungi and wort agar. The number of colony-forming units (CFU) was counted manually; pure cultures were isolated from the plates for further identification.

The bacterial cultures were identified by their morphological, physiological, and biochemical characteristics (*Bergey's Manual...*, 1993; Lysak et al., 2003).

To determine the species status of the dominant bacteria, we used the technique of sequencing the nucleotide sequence of *16S* rRNA genes. DNA extraction was performed using the standard phenol–chloroform method.

Amplification was performed on a Mastercycler thermocycler (Eppendorf, Germany) using EUB f933 and EUB r1387 primers of the following form:

5'-GCACAAGCGGTGGAGCATGTGG-3',

5'-GCCCGGGAACGTATTCACCG-3', respectively.

The annealing temperature was 60°C, and the duration of the denaturation, annealing, and elongation was 30 s for 27 cycles. The sequencing of the amplicons was carried out using the Sanger method (Syntol, Moscow). The sequences were analyzed using the PubMed electronic database with the BLAST2 algorithm. In the sequencing, a strain often matched several species (when a strain is mentioned for the first time in this paper, the possible variants of species will be given in brackets).

To evaluate the functional diversity of the soil, wood, and intestinal microbial communities, the multisubstrate test (MST) was used (Gorlenko and Kozhevin, 2005). For the soil and wood, samples weighing 0.7 g were taken, for the contents of the intestine, we used a mixed sample of 3–5 larvae (0.01 g). The test suspension was increased in volume to 35 mL with phosphate buffer (pH 6.5), processed on the VORTEX V-3 laboratory shaker for 1.5 min and then centrifuged for 2 min at 2000 rpm (CUM-8, USSR).

Table 1. Total number of bacteria in the intestine of wireworms and their habitats (according to the data of fluorescent microscopy)

Substrate	Number of bacterial cells, $\times 10^9$ per 1 g	Total length of fungal hyphae, m/g
Soil	1.7	120
Wood	7.5	6000
Agriotes obscurus	0.01–0.15	–
Selatosomus aeneus	0.03–0.15	1.5–2
Ampedus pomorum	0.015–0.05	0–3

First, 20 mL of the supernatant was placed in the cuvette of a dispenser, then 2 mL of indicator solution (triphenyltetrazolium) was added. The contents of the cuvette were dispensed into a test plate (Eco-Log, Moscow) with a set of 47 test substrates with an eight-channel dispenser (Eppendorf) with disposable tips set to dispensing 200 μL. The plates were incubated in a thermostat at 28°C until the appearance of a visually detectable staining of the cells (~72 h).

The optical density (OD) of the plates' cells was measured using a slide scanner. The intensity of substrate consumption (color of each cell) ranged from 400 to 4500. The data set obtained by the photometric measurement of the OD values for all cells represents a range of substrate consumption (RSC) for the studied soil microbial complex.

When analyzing the RSC, the Eco-Log software was used, which allows us to calculate automatically the traditional biodiversity parameters (H' is Shannon index, E is evenness, N is the number of the consumed substrate, and W is the specific metabolic work), the coefficient of stability of the microbial community (d), and the coefficients of rank distributions of the ranges of substrate consumption. The microbial communities are divided into prosperous redundant systems, with a maximum safety margin ($d = 0.01–0.1$); stable ones ($d = 0.1–0.4$); systems with resource depletion or under the reversible influence of a disturbing factor ($d = 0.4–0.8$); critically destabilized systems ($d = 0.8–1$); and irretrievably broken systems, which lost their functional integrity ($d > 1$).

RESULTS

The total number of microorganisms of the larvae intestine. According to fluorescent microscopy, the number of bacterial cells in the intestine of wireworms was 1–2 orders of magnitude lower than in the humus horizon of the soil and decaying wood (Table 1). At the same time, in the larvae of all species studied, the numbers of bacteria were of the same orders. The hyphae of fungi in the intestine of the larvae were rare. This also applies to *A. pomorum*, which inhabits wood with a very high abundance of micromycetes.

In the intestine of the soil larvae, the ratio of physiologically active and inactive cells was determined compared to that of the soil. In the soil, the number of inactive cells was $3.38 \pm 0.54 \times 10^9$ g and that of active was $0.424 \pm 0.068 \times 10^9$ g (ratio 8 : 1). In the intestine of larvae, the ratio of inactive/active cells was reverse: 1 : 25 in *A. obscurus* ($0.03/0.75 \times 10^9$ per 1 g) and 1 : 12 in *S. aeneus* ($0.09/1.11 \times 10^9$ per 1 g). Thus, in the soil, only an eighth of the bacterial cells is physiologically active and identified by EUB338-mix probes, whereas in the intestinal contents, they are an order of magnitude higher than the number of inactive cells.

Among the physiologically active cells in the intestine, an important role is played by Archaea (Table 2), the number of which is higher by an order of magnitude than in the soil habitats of the wireworms. In *A. obscurus*, archaea predominate by the number of cells, while in *S. aeneus* they constitute ~40%. As part of the eubacterial population, the groups of Actinobacteria and γ-Proteobacteria are numerous. Actinobacteria are widely distributed in soil and water communities, the majority of them are aerobes involved in the decomposition of cellulose and chitin. γ-Proteobacteria include Enterobacteriaceae and Pseudomonas. Among them, there are many symbiotic forms associated predominantly with aquatic invertebrates.

In the soil samples studied, actinobacteria accounted for approximately one-third of the total number of the active bacterial community, whereas the abundance of γ-proteobacteria was lower by an order of magnitude. In the intestine of the soil larvae, the abundance values of actinobacteria and γ-proteobacteria were almost identical (1 : 1). In sum, the abundance of both groups in the intestine was higher than that in the soil.

Determination of the composition and abundance of the saprotrophic forms. In the inoculations on GPA, the CFU of saprotrophic forms in the soil and wood were of the same order of magnitude (1.1×10^7 and 1.7×10^7 CFU/g, respectively). In *A. obscurus*, the widest range of abundance variations was observed ($1.8–3.5 \times 10^7$ CFU/g). In *S. aeneus* and *A. pomorum*, the total number of saprotrophic intestinal microorganisms was $0.57–2 \times 10^7$ and $0.12–1 \times 10^7$ CFU/g, respectively.

The taxonomic composition and structure of the saprotrophic bacterial population in the larvae intestines and their habitat were drastically different. The

Table 2. Ratio of physiologically active cells ($\times 10^9$ per 1 g) in the soil and intestine of wireworms (according to the FISH data)

Groups	Soil	*Agriotes obscurus*	*Selatosomus aeneus*
Eubacteria	0.37 ± 0.07	0.34 ± 0.26	0.67 ± 0.14
actinobacteria	0.1 ± 0.02	0.32 ± 0.12	0.36 ± 0.01
γ-proteobacteria	0.06 ± 0.02	0.3 ± 0.14	0.37 ± 0.06
Archaebacteria	0.058 ± 0.01	0.41 ± 0.09	0.44 ± 1

Table 3. Composition of the saprotrophic bacterial population in the larvae intestine and their habitats ($\times 10^6$ CFU/g of suspension)

Groups of microorganisms	Soil	Larvae intestine		Wood	Intestine of *Ampedus pomorum*
		Agriotes obscurus	*Selatosomus aeneus*		
Streptomyces spp.	1.67	0.95	–	1.077	1.9
Bacillus spp.	4.94	0.1	–	4.5	0.19
Myxococcales	0.61	6.27	0.28	4	0.19
Cytophaga spp.	–	–	–	5.7	–
Gluconobacter spp.	–	–	0.63	–	–
Gram-positive cocci	0.04	2.85	0.19	–	2.09
Enterobacteriaceae	0	3.8	2.85	0	0
Aquaspirillum spp.	0.38	0	2.85	–	–
Vibrionaceae	–	–	0.19	–	–
Acinetobacter spp.	–	1.33	3.8	–	–
Coryneform bacteria	2.77	13.3	2.28	0.19	0.66
Nocardioides plantarum	–	–	0.25	–	–
Erwinia spp.	0.3	0.76	0.38	–	0.08
Xanthomonas spp.	–	0.19	0.04	–	–
Mesorhizobium amorphae	–	–	1.07	–	–
Total	10.71	25.75	14.81	15.657	5.68

"–" not found.

soil was dominated by groups of the hydrolytic complex: streptomycetes, bacilli, myxobacteria, and coryneform bacteria. In the wood, the saprotrophic microflora was depleted in comparison with the soil, although the total number was 1.5 times higher. The wood contained all the groups of the hydrolytic complex found in the soil, as well as bacteria of the genus *Cytophaga*, which prevailed in the bacterial community.

In the intestine of *A. obscurus*, myxobacteria and coryneform bacteria were numerous among the soil groups, and their absolute numbers were higher than in the soil by an order of magnitude (Table 3). In the soil, the coryneform bacteria were represented by *Corynebacterium* spp., and in the intestines of the soil larvae, they were represented by the genera that were not found in the soil.

In *A. obscurus*, *Microbacterium paraoxydans*, *M. oxydans,* and *Tsukamurella tyrosinolvens* (or *T. pulmonis*) dominated. These larvae also had a high abundance of bacteria that are typical of the animal intestine (8×10^6 CFU/g) represented by cocci, *Acinetobacter* spp. and Enterobacteriaceae. Cocci were found in the soil as well in a small amount, whereas the latter two groups were absent. The intestines also contained *Xanthomonas* spp. and *Erwinia* spp., which are found on vegetation.

In the intestine of *S. aeneus*, the total number of saprotrophic bacteria was two times lower than that of *A. obscurus*. Streptomycetes and bacilli were absent, the amount of myxobacteria was insignificant. The abundance of coryneform bacteria was almost the same as in the soil, among them, *T. tyrosinolvens* dominated. Among the intestinal forms, apart from cocci, *Acinetobacter* and Enterobacteriaceae, representatives of Vibrionaceae were found, which were absent in the soil and in *A. obscurus*. In *S. aeneus*, the group variety of the intestinal microorganisms was higher than in *A. obscurus*; in particular, *Gluconobacter* was found,

Table 4. Parameters of the functional diversity of the microbial complexes

Parameters	*N*	*W*	*E*	*H'*	*d*	*G*
Agriotes obscurus	32–34	1980–2657	0.983–0.993	4.914–5.05	0.89–0.359	95.72–222.84
Selatosomus aeneus	20–32	1427–2624	0.981–0.985	4.24–4.93	1.769–0.6	28.26–133.3
Ampedus pomorum	18–24	1299–1322	0.955–0.986	4.1–4.52	1.79–1.785	21.3–33.61
Soil	21	2356.05	0.96	4.217	0.7095	74.05
Wood	37	3162.24	0.986	5.14	0.04	2055.56

(*N*) Number of consumed substrates, (*W*) average intensity of substrate consumption, (*E*) evenness, (*H'*) Shannon index, (*d*) stability parameter, and (*G*) integral vitality index.

which is associated with the sites of decomposition of sugary foods and is found in the soil in small quantities, as well as inhabitants of plant tissues such as *Nocardioides plantarum* and *Mesorhizobium amorphae* and water-based *Aquaspirillum* spp.

In the intestine of the wood predator *A. pomorum*, the abundance of the saprotrophic microflora was three times lower than in the wood. It did not include any *Cytophaga* spp., in the hydrolytic complex. Only streptomycetes were present. In the wood, the intestinal forms were missing, while in the intestine of the larvae, they amounted to 2.1×10^6 CFU/g. Among them, cocci prevailed and enterobacteria were found. Coryneform bacteria in *A. pomorum* were not determined to the species level.

In the nitrogen-free Ashby medium, the number of nitrogen-fixing bacteria was determined. In the soil larvae, the composition of the complex of nitrogen-fixing bacteria was similar. A monodomination of coryneform bacteria *M. oxydans* was observed. In *S. aeneus*, the total number of bacteria on the Ashby medium reached 10^8 CFU/g.

In the wood *A. pomorum*, the total number of nitrogen-fixing bacteria was significantly lower ($1.2–3.6 \times 10^5$ CFU/g), and the taxonomic structure was fundamentally different from that of the soil larvae: the bacilli and Gram-positive cocci were dominant (*M. luteus*, etc.).

The diversity of the bacterial intestinal complexes was assessed using *H'*. In *A. obscurus* and *S. aeneus*, *H'* = 3.1 and 3.2, respectively, while in the soil it was 3.7, and in the wood predator *A. pomorum*, 2.25, i.e., significantly lower than that of the soil larvae, but two times higher that of the wood (1.4).

Determination of the abundance of micromycetes. In the wood and the intestine of the *Ampedus* larvae, the composition and abundance of micromycetes were determined. The wood contained nine species, three of which occurred sporadically in the intestine of wireworms: *Trichoderma harzianum, Aspergillus clavatus,* and *Penicillium citrinum*. No others micromycetes, apart from those that presumably came from the wood, were found in the wireworms. In the wood, the number of fungi isolated on the Czapek mineral medium was 8000, and on wort agar, 19280 CFU/g, whereas in the intestine of the larvae, it was lower by an order of magnitude.

The structure of domination of the intestinal bacterial complexes. Figure 1 shows the ratio of the major groups in the intestinal bacterial complexes of wireworms and their habitats. The soil was dominated by the hydrolytic groups and coryneforms, in the intestine of *Agriotes*, a large proportion of the intestinal forms was added. In *Selatosomus*, the relative abundance of different groups of microflora was more aligned: a significant role was played by the microorganisms associated with plants and aquatic habitats. The total number of intestinal microflora in *Selatosomus* was more than two times lower than in *Agriotes*. The wood was dominated by the groups of the hydrolytic complex, which, in addition to the soil forms, included *Cytophaga*, and in the intestine of *Ampedus*, where the total abundance of saprotrophic bacteria was the lowest, half of the population consisted of bacteria typical of the intestine of invertebrates.

Evaluation of the functional diversity of intestinal microbial complexes. To evaluate the functional capacity of the intestinal microbial communities using the MSD method, 46 standard substrates comprising 14 amino acids, 3 nitrogen-containing compounds, 3 polymers, 5 alcohols, 8 organic acid salts, and 13 sugars were used. The functional diversity and stability of the intestinal microbial communities of the larvae increased in the series *A. pomorum–S. aeneus–A. obscurus* (Table 4). The minimum values of the parameters of the functional diversity and stability were typical of the intestinal microbial community of wood larvae, while in the wood these parameters reached maximum values.

The OD of the cells, which is an indicator of the activity of substrate consumption, ranged from 400 to 4500. Four degrees were distinguished on this scale: (I) <1200, (II) 1400–2000, (III) 2200–3000 and (IV) >3200. We compared the activity of consumption of the most numerous groups of organic compounds by the intestinal microbial communities of three wireworm species (Fig. 2).

Of the 14 amino acids tested, the bacterial complex of *Agriotes* consumed all the substrates; *Selatosomus* and *Ampedus* consumed 13 and 9 amino acids, respec-

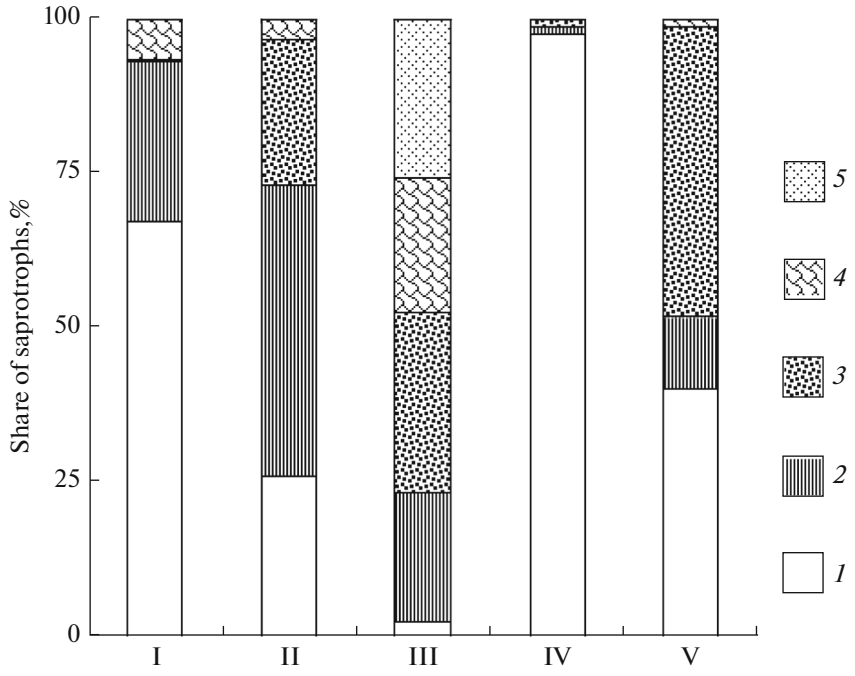

Fig. 1. The structure of domination in the intestinal bacterial complexes. (*1*) Hydrolytic complex, (*2*) coryneform bacteria, groups of microorganisms typical of (*3*) the animal intestine, (*4*) plants, and (*5*) aquatic habitats. (I) Soil; (II, III, and V) intestines of *Agriotes*, *Selatosomus*, and *Ampedus*, respectively; (IV) wood.

tively. In *Agriotes,* bimodal activity for different amino acids with peaks in the regions of the highest and lowest degrees of consumption was observed. In *Selatosomus*, the degree III was registered for most of the amino acids, whereas in *Ampedus*, the lowest activity of amino acid intake was noted. Thus, *Ampedus* showed the narrowest range and the lowest amino acid consumption activity, which may be related to its predation. In *Agriotes*, a certain selectivity for individual amino acids can be noted, in particular, histidine, glutamine, serine, asparagine, and proline, which are consumed with the maximum activity, and norleucine and glycine, which are consumed with the lowest activity. The last two amino acids are not consumed by the bacterial complex of *Ampedus*. In *Selatosomus*, a high degree of activity was observed only for proline and arginine.

Of the eight salts of organic acids tested, the bacterial complexes of *Agriotes* and *Selatosomus* consumed all substrates, whereas *Ampedus* consumed only six (not maleate and octanoate). In the intestine of *Agriotes*, the maximum consumption rates were observed for aspartate and maleate, and the minimum ones, for octanoate. The other organic acid salts were consumed with an average activity. In *Selatosomus*, a high activity was observed in relation to maleate, okatnoata, and propionate, and minimum activity was noted for succinate. In *Ampedus*, salt intake ranged from 2200 to 3200 OD, minimal consumption was noted for succinate, citrate, and acetate. Thus, the selectivity for the salts of organic acids was similar in the bacterial complexes of the soil larvae, while in the wood *Ampedus* an alternative trend was observed.

All tested sugars were consumed by the bacterial complexes of the three wireworm species. *Ampedus* displayed the highest activity in consuming most of the sugars, in *Selatosomus*, selectivity toward individual forms was clearly expressed. For example, the rate of consumption of fructose, arabinose, mannose, and glucose was high, while that of raffinose, lactose, rhamnose, and sucrose was low. In *Agriotes*, most of the sugars are consumed with degree II of activity.

A similar consumption of the main groups was observed in the intestinal microbial communities of *S. aeneus* and *A. obscurus*. Exceptions were oligosaccharides and amino acids, which are more actively consumed by the microflora of *A. obscurus*, while nitrogen-containing compounds are consumed more actively in the intestine of *S. aeneus*. The microbial community of the intestine of *A. pomorum* consumes all groups of substrates with the exception of polymers (starch, tween, dextran) much less intensively. Only three of the substrates were consumed intensively in all the samples studied: aspartate, glutamate, and lactate.

DISCUSSION

The composition of the intestinal microflora of soil invertebrates was studied mainly in the representatives of saprophages feeding on litter, soil detritus, and

wood. The bacterial population of their intestines is characterized by high abundance, as it includes both the forms consumed with food (and used as food) and the native population of the intestine. In diplopods, the number of saprotrophic forms was 2.8×10^9, and in woodlice, the number of proteobacteria was 2.7×10^8 CFU/g (Byzov, 2005). In herbivorous insects, the population numbers of the intestinal bacteria was lower. For example, the number of bacteria in the intestinal contents of the Colorado potato beetle larvae was ~10^6 cells/g (Shivokene and Malukas, 1988). Wireworms occupy an intermediate position: the number of saprotrophes in their intestine is ~10^7, and their total number is 10^8.

The composition of the microbial population in the intestine of pedobionts is fundamentally different from that in their food, which was shown for saprophages (Reyes and Tiedje, 1976; Anderson and Bignell, 1980). The main difference is the absence or low numbers of micromycetes (Schönholzer et al., 1999), which serve as food for many detritophages and bacteriophages. In the soil larvae of *A. obscurus*, no fungal mycelium was found in the intestine, while in the intestine of the larvae of *S. aeneus* and *A. pomorum*, small pieces of mycelium were observed. It should be noted that in the soil recycled by the larvae of Elateridae, the fungal hyphae were completely damaged (Samoilova et al., 2015b). It is possible that wireworms use mycelium as food.

The taxonomic composition of intestinal bacteria in the investigated wormwood species was significantly different from the microflora of the environment. More than half of the saprotrophic bacterial cells in the larvae were represented by groups absent in the soil and wood, and the intestinal bacterial complexes included specific intestinal forms. According to the Bray–Curtis index, the similarity of composition of the soil microflora and intestinal community in *A. obscurus* is 0.23 and in *S. aeneus*, 0.25; the similarity of the microbial community of the wood and intestines of *A. pomorum* was 0.16. In *A. obscurus*, the share of intestinal forms amounted to 31% of the total; among them prevailed cocci and enterobacteria. In *S. aeneus*, the share of intestinal groups reached 67% with the dominance of Enterobacteriaceae and *Acinetobacter* spp. Furthermore, in both species a significant role was played by the coryneform bacteria represented by species that were absent in the soil. In this case, the proportion of native forms in the intestinal bacteria complex exceeded two-thirds of the total abundance. In the intestine of *S. aeneus*, high numbers of *Aquaspirillum* spp. were found, which are water forms that can develop under anaerobic conditions and use nitrate instead of oxygen. In the wood predator *A. pomorum*, with a very low number of saprotrophic bacteria, 37% were intestinal forms presented by cocci.

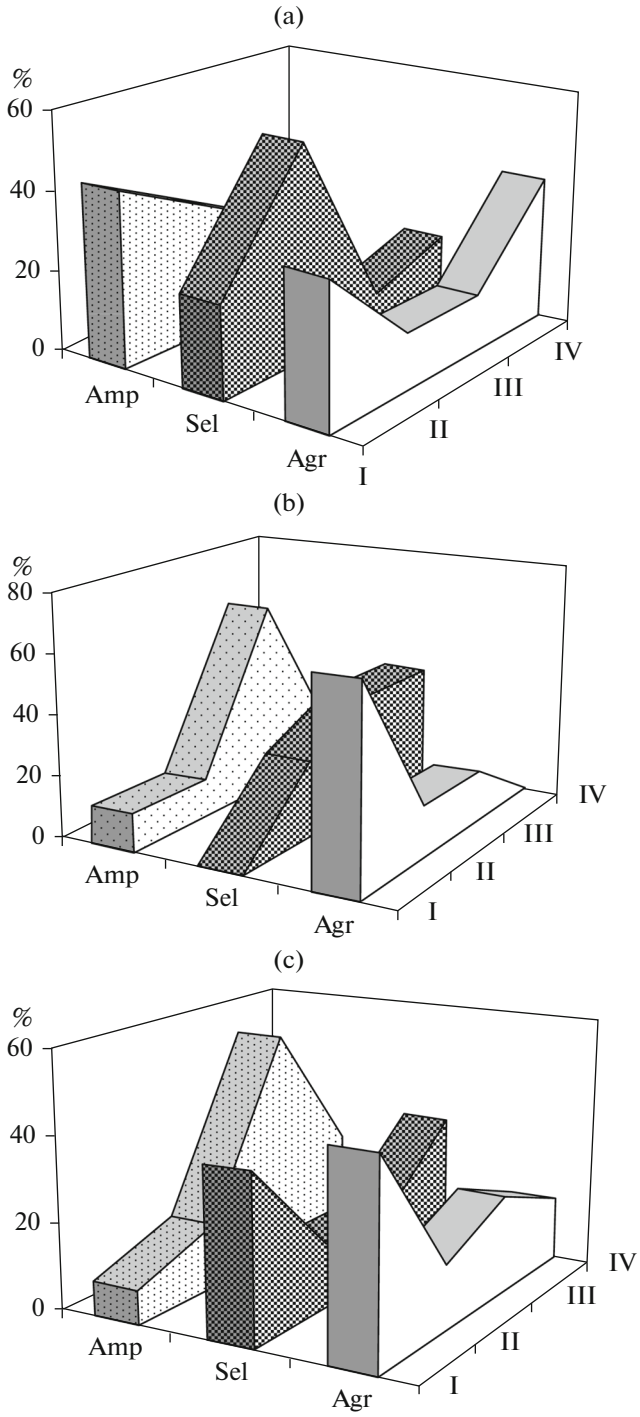

Fig. 2. Variability of the degree of consumption of various groups of organic compounds by the bacterial complexes of the intestine ((a) amino acids, (b) salts of organic acids, and (c) sugars). (Amp) *Ampedus*, (Sel) *Selatosomus*, and (Agr) *Agriotes*. Activity of substrate consumption (by the optical density): (I) <1200, (II) 1400–2000, (III) 2200–3000, (IV) >3200.

Thus, the intestinal microbial complexes of wormwood larvae include specific intestinal groups, as well as free-living forms, which transit to life in the intestine of pedobionts with respiration and nutrition con-

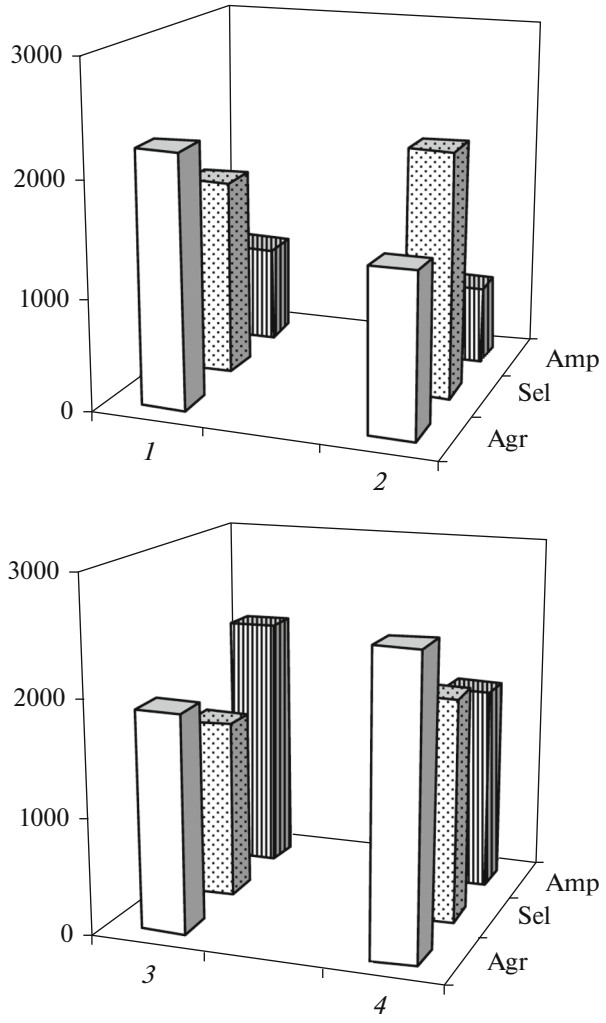

Fig. 3. Average rate of (*1*) amino acids, (*2*) nitrogen-containing compounds, (*3*) polymers, and (*4*) sugar consumption.

ditions optimized for them. The transit of microorganisms from the soil and water to the intestine of soil animals has been repeatedly noted in saprophagjus invertebrates. For example, in the northern areas of the central part of Russia, *Aspergillus fumigatus* was found in the intestines of earthworms, which freezes in this region in the winter soil (Striganova, 1995). In the intestine of diplopods, a high number of *Promicromonospora* spp. was observed, which occurred sporadically in the soil (Szabó et al., 1985). It is evident that the Elateridae larvae intestine is also a fertile habitat for a number of groups of microorganisms, especially in open habitats with regular periods of water deficit in the soil. In the intestine, these forms are involved in digestion.

The group composition and functional characteristics of the intestinal bacteria of Elateridae larvae have distinct species differences determined, most likely, by the difference in the diet modes of larvae. The bacterial complexes of the soil larvae of both studied species are characterized by a high activity in the consumption of nitrogen-containing compounds. For *A. obscurus*, the maximum consumption of amino acids was registered, and for *S. aeneus*, that of other nitrogen-containing compounds (Fig. 3). In the wood *A. pomorum*, the intensity of consumption of nitrogen compounds was almost two times lower. For polymeric compounds, an inverse correlation was observed: the highest average OD values were observed in *A. pomorum* and considerably lower ones were found in the soil species. Sugar consumption in all species was high, and the maximum rate of consumption was observed in *A. obscurus*. Thus, soil larvae are the most actively involved in the transformation of nitrogen-containing compounds and the wood *A. pomorum* exhibits high activity in polymer degradation. The intestines of the latter contain a relatively high content of streptomycetes, which are involved in chitin degradation. Apparently, its predation is combined with the ability of the intestinal microbial population to conduct hemicellulose degradation and mineralization of organic matter. This confirms the mixed diet mode: combination of predation with xylophagy.

The high number of nitrogen-fixing bacteria in the intestine of wireworms observed by us confirms the high nitrogen fixation activity found previously, which exceeds that in the soil by 2–3 orders of magnitude (Samoilova et al., 2015a). This, in turn, may explain the high activity of transformation of nitrogen-containing compounds by the complex of intestinal bacteria. In the forest soil, recycled by the Elateridae larvae inhabiting it, increasing numbers of microflora associated with nitrogen-containing compounds, in particular, ammonifiers, were also found (Kozlovskaya, 1976). Active participation in the transformation of nitrogen-containing compounds is combined with a high rate of consumption of mono- and oligosaccharides and alcohols, which are the main energy source (Fig. 3).

The composition of the intestinal microflora of soil larvae of *Agriotes* and *Selatosomus* shows the differences in their feeding activity. In the larvae of *A. obscurus*, a significant part of the active bacterial population is represented by soil forms, the total number of which in the intestine is an order of magnitude higher than in the soil. At the same time, the number of bacteria that are typical of plant substrates is negligible. This suggests that, in the studied biotope, the larvae of *A. obscurus* use detritus soil as food. The soil microorganisms, entering the intestine with food, are a transit component. Bacteria from the order Myxococcales, which are typical soil forms producing proteases and cellulases and actively destroying organic compounds, are especially abundant in the intestine of *A. obscurus*.

The combination of phytophagy and detritophagy is known for many members of Elateridae, which damage field cultures. In field experiments, transition

to detritophagy was shown in the conditions of sufficient soil moisture for *Agriotes* larvae (Gilyarov, 1949). In this case, the larvae were collected from the floodplain soils with sufficient moisture, so their preference for the soil detritus rich in semi-decomposed organic matter is quite likely.

In the *S. aeneus* larvae, the population of soil forms is very low in the intestinal microflora and, at the same time, the diversity of bacteria associated with plants is relatively high: their total share in the community is three times higher than in the soil. This indicates a preference for phytophagy along with predation. The latter is confirmed by the high splitting activity of nitrogen-containing compounds (Fig. 3).

The intestinal microflora of wireworms was studied in only a few species. In the larvae of *A. lineatus* from the Black Sea coast of eastern Turkey, 19 strains of bacteria belonging to 12 genera were identified (Danismazoglu et al., 2012). Most of them had previously been isolated from insects, and only two species were isolated from the intestines of insects for the first time (*Arthrobacter gondensis* and *Pseudomonas plecoglossicida*). In our material, in all larvae species, representatives of 11 genera of bacteria were identified, of which 7 were found in the intestine of *Agriotes*.

A relatively high similarity of the bacterial complexes of the wireworms studied by us and Elateridae larvae from Costa Rica was revealed (Gonzales, 2009): in the lower intestine of the larvae, γ-proteobacteria, actinomycetes, and representatives of Fimicutes dominated. In total, 11 cultures of bacteria were identified, including four species of bacilli, enterobacteria, *Acinetobacter* spp., and *T. pulmonis*. Thus, the taxonomic composition is similar to that observed in the investigated wireworm species.

We also studied in detail the intestinal microflora of the *Limonius canus* larvae, which is a potato pest in Oregon (Lacey, 2007). Fifteen genera of bacteria were identified, of which *Erwinia* and *Acinetobacter* were in common with our material. The intestine of *Limonius* was dominated by *Bacillus megaterium* and *Ranella aquaticus*. The latter belongs to the group of γ-proteobacteria, which are also numerous in the larvae of Costa Rica and the larvae from the soil of the Russian steppes.

The high α- and β-diversity of the intestinal bacterial complexes in all studied species of click beetles larvae should also be noted. The similarity of the bacterial population is manifested only at the level of high taxa. It remains unclear whether the differences in the generic and species composition of the intestinal microorganisms are a consequence of the geographical remoteness of certain populations or are determined by the biotopical and dietary adaptations. The wide geographical variations in the composition of the intestinal bacterial complexes in representatives of the same species could possibly depend on the differences in preferences for different groups of plants.

The intestine of insects is an attractive niche for the settlement of microorganisms, which, in the course of evolution, has stimulated the development of symbiotic relationships (Dillon, R.J. and Dillon, V.M., 2004). The microbial transformation of complex organic compounds in the insect intestine allowed them to assimilate a wide range of suboptimal resources, in particular, the structural components of plant tissues. Among insects, groups with varying degrees of development of symbiotic relationships with microorganisms are distinguished. Xylophages and some soil saprophages have developed complexes of symbiotic organisms, obligate anaerobes, which form intestinal food chains (termites, cockroaches) and provide the owners with energy, vitamins, and essential amino acids (Bignell, 1984). In a number of forms, the intestines have developed special morphological structures, in which endosymbionts are concentrated (Tipulidae, Anobiidae, and Cerambycidae) (Breznak, 1982). At the same time, many of the soil saprophages with a simple tubelike intestine (diplopods, woodlice) have no strict anaerobes, and most microorganisms are facultative anaerobes (Reyes and Tiedje, 1976; Dobrovol'skaya, 2002). In diplopods and earthworms, even the parietal community has facultative anaerobes (species *Klebsiella* and *Enterobacter*) (Tretyakov et al., 1996; Byzov et al., 1998). In Elateridae larvae, all intestinal forms are facultative anaerobes. This indicates a certain "youth" of the symbiotic relationships of wireworms with the soil microflora in the process of their adaption to phytophagy.

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