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Are chitinolytic bacteria present in the gut of Glomeris hexasticha (Diplopoda)?

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Abstract. The hind gut of Diplopoda is rich in chitinous structures protruding into the lumen, which offer many sites for colonization of intestinal bacteria. Only very few scattered data are available in the literature on the occurrence of chitinolytic bacteria in the gut of soil invertebrates including millipedes, too. Studying the taxonomic composition of aerobic and facultatively anaerobic microbiota in the hind gut or fresh faecal matter of *Glomeris hexasticha*, we selected altogether 121 representative strains of the most important gut colonizing nocardioform actinomycete, *Pseudomonas*, *Chromobacterium*, *Flavobacterium*, etc. species. Neither from among these strains proved to be able to hydrolyse rapidly raw chitin. But 15 strains from among the 121 studied ones proved to be able to decompose partially hydrolysed chitinous preparates. In our opinion during the first selection processes taking place among the pioneer invaders in the gut of the young millipedes strongly active raw chitin-decomposers cannot attain a community position.

As it is known, chitinolytic bacteria occur frequently in lake and river muds, soils, etc. where chitin-containing materials from dead insect bodies desintegrated fungal hyphae etc. are intensely decomposed. Very few information are, however, available on the cocurrence of such microbes in the gut of soil invertebrates. Studying the intestinal microbiology of millipedes we demonstrated (Dzingov et al., 1982; Szabó et al., 1983; Márialigeti et al., 1985; Chu et al., 1987, etc.) many types of bacteria and their physiological abilities, which can colonize the hind gut of these animals. Since the hind gut of millipedes is rich in bristle-like chitinous structures functioning among others in the disruption of the peritoneal membrane, the question arouses: are chitin-decomposers present in this intestinal milieu, where the chitinous bristles play in the digestion an important indirect part? Below we try to answer on this question in the case of the millipede *Glomeris hexasticha* common in Hungarian forest soils.

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Materials and methods used

From aseptically collected, mixed faecal matter of different *Glomeris hexasticha* adult specimens a standard suspension and a dilution series were prepared, and plated on the surface of agar media of different composition. Altogether 1435 isolates were obtained. From among the latters 121 strains representing different types and species of gut bacteria were selected for detailed, computer aided taxonomic analyses (the results are presented in the work of CHU, 1986). These were tested also for chitinolysis. For this purpose the medium of JOHNSTON and CROSS (1976) was used: colloidal (or pulverised raw) chitin 2.0 g; $FeSO_4 \cdot 7H_2O$ 0.01 g; KCl 1.71 g; $CaCO_3$ 0.02 g; $MgSO_4 \cdot 7H_2O$ 0.05 g; Na_2HPO_4 1.63 g; agar 18.0 g; distilled water 1000 ml; pH 7.2. Colloidal chitin was prepared according to LINGAPPA and LOCKWOOD (1962). The inoculated plates were incubated at 28°C for 7 to 14 days. The chitinase production was indicated by clearing of the opaque medium.

Results and discussion

The tested 121 representative bacterial strains were indentified as members of different genera and species. 10 belonged to a new type of true nocardioform intestinal actinomycetes (CHU et al., 1987). 15 strains proved to be members of a *Klebsiella* sp. 51 strains, representing the predominant, indigenous gut bacterial fraction, were identified as organisms showing considerable similarity to *Pseudomonas stutzeri*, but not correctly identifiable with this species. 6 strains were identified at generic level as *Chromobacterium*, etc.

Among these strains many showed positive casein, aesculin, Tween–80, DNA, starch, gelatin, tributirin, partially degraded chitin etc. hdrolysis, hypoxanthine, xanthine, tyrosine, arbutin, etc. decomposition (see Table 1). But cellulose and raw chitin remained in all cases unattacked by them. We suppose that strongly active raw chitin decomposers do not develope in the hindgut of *Glomeris hexasticha*. Such organisms, presumedly, disappeare already

Table 1. Decomposition of some selected organic compounds by representative gut bacterial strains of Glomeris hexasticha

	Numbers of strains	
	positive	negative
Cellulase production	0	121
Decomposition of raw chitin	0	121
Decomposition of partially hydrolysed chitin	15	106
Urease production	116	5
Starch hydrolysis	71	50
Aesculin hydrolysis	115	6
Casein hydrolysis	87	34
Gelatin hydrolysis	85	36
Tween 80 hydrolysis	110	11
Xanthine decomposition	17	104
Hypoxanthine decomposition	64	57
Testosterone decomposition	16	105

during the pioneer colonization of the intestinal lumen by bacteria and cannot attain community position in the hindgut biota. This seems to be evident if we are considering the fact that the decomposition or destruction of the chitinous intestinal structures of millipedes could influence disadvantageously the digestive processes.

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