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Microanatomical and biological aspects of bacterial associations in *Tyrophagus putrescentiae* (Acari: Acaridida)

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Abstract. The occurrence of bacterial colonies in the mesenchymal tissue of *Tyrophagus putrescentiae* Schrank was studied. One algal and two fungal species were offered as food. The experiment was analysed histologically and via transmission electron microscopy and plating of the mite homogenate. The dominant bacterium species, the bacterium population density in the mesenchyme, the location of bacterium groups between the internal organs and the ultrastructure were investigated. There were conspicuous differences between food types tested. On *Penicillium*, the highest population of bacteria in the mesenchymal tissue was correlated with the chitinase activity in mite homogenate. In this homogenate, *Serratia marcescens* was highly dominant among the other plated bacteria and it exhibited strong chitinolytic and trehalolytic activity.

Introduction

The bacteria in the body of soil arthropods have been studied thoroughly in diplopods (Anderson and Bignell 1980; Crawford et al. 1983). Highly diversified communities were reported inside the diplopod gut, mainly by scanning electron microscopy (SEM). In oribatid mites Hartenstein (1962), Seniczak and Stefaniak (1978) and Stefaniak and Seniczak (1976, 1981) plated the internal microbial communities. Wolf and Rockett (1984) dissected oribatids and obtained their internal microflora. All papers correlated the micro-organisms with food – as part of ingested food or producers of some enzymes. Extra-intestinal groups of micro-organisms were reported in soil saprophagous mites (Oribatida and Acaridida) by Smrž and Čatská (1989), Smrž and Trelová (1995), Smrž et al. (1991) and Smrž (1996, 1998). They occurred in several mites (*Tyrophagus putrescentiae, Damaeus auritus, Damaeus riparius, Scutovertex minutus*), forming loose groups or well-defined bodies. Such bodies have also been noted in *Chamobates voigtsi* (Černý 1991, 1999) and *Tectocepheus velatus* (Hajmová 1997), both from field samples. Luxton (1972, 1991) discussed the role of bacteria in the nutritional biology of mites. Smrž

et al. (1991) and Smrž (1996, 1998, 2000) suggested a substantial role for some associated bacteria in the digestion of some compounds (chitin, cellulose).

This paper studies the correlation of qualitative characteristics of internal associated bacteria with the type of ingested food. These bacteria form groups in the mesenchymal tissue between the mesenchymal cells between internal organs, hence, outside the gut. The ultrastructure of these groups is described.

Materials and methods

Tyrophagus putrescentiae was used as a model species for food selection experiments, since it is able to consume many types of food. Its reproduction is very rapid and it has been reared in our laboratory for many years and used for such purposes (Smrž and Čatská 1987, 1989; Smrž and Trelová 1995; Smrž et al. 1991). Our mite population originates from the alfalfa field near the city of Praha (Smrž and Jungová 1989). Two fungi (*Penicillium* sp. and *Alternaria alternata*) from our collection were offered as chitin-rich food. These fungi were grown on malt agar (pH 6.8) in Petri dishes and offered separately (not as a cafeteria test) to mites. For comparison, the alga *Protococcus* sp. from apple tree bark was offered as food without chitin. The bark fragments were placed into glass jars with a plaster of Paris/charcoal bottom. All these experiments were triplicated at laboratory temperature (20–22 °C) and air humidity (50–60%). Approximately 60 mites per dish or glass jar (adults and tritonymphs) were introduced and subsequently sampled after one day (10 individuals), three days (5 individuals from algae) and five days (10 individuals from fungi).

Ten sampled specimens were fixed in Bouin-Dubosque-Brasil modified for soil mites (Smrž 1989), sectioned on the Leica 2155 rotation microtome (section thickness 5000 nm) and stained in Masson's triple stain before being observed with a Provis AX 70 microscope (Olympus) with editing of microphotos in the Microimage 3.1 image analysis (Olympus). The application of Nomarski DIC under the microscope and inversion of the image in image analysis was very useful.

For transmission electron microscopy (TEM), five mites were fixed in cacodylate-buffered glutaraldehyde (4%), postfixed in 1% osmium tetroxide, embedded in Spurr medium and sectioned with an Ultracut ultramicrotome (Reichert). Sections were stained in lead citrate and uranyl acetate and observed under a Philips EM 300 microscope (TEM).

The internal bacteria from the mite homogenate (10 mites pooled due to their small size) were plated using the technique of Smrž et al. (1991) and identified in the internationally certificated microbiological institute (CCM Brno, Czech Republic). The chitinase activity of the same homogenate of mites was tested on a carboxymethylchitin layer with subsequent staining by basic fuchsin (Smrž 2000), trehalase activity of *Serratia marcescens* was revealed by CCM in their analyses. Both enzyme activities of *Serratia marcescens* was reported in our previous papers (Smrž

and Čatská 1987, 1989; Čatská et al. 1989; Smrž and Trelová 1995; Smrž et al. 1991).

The results should detect presence or absence of bacteria along with enzyme activity. No quantitative correlations were established. In bacteria plating, the species dominance was very clear. The actually dominant bacterial species formed over 10 CFU (colony forming units) per dish. Other identified bacteria, formed 1–2 CFU per dish.

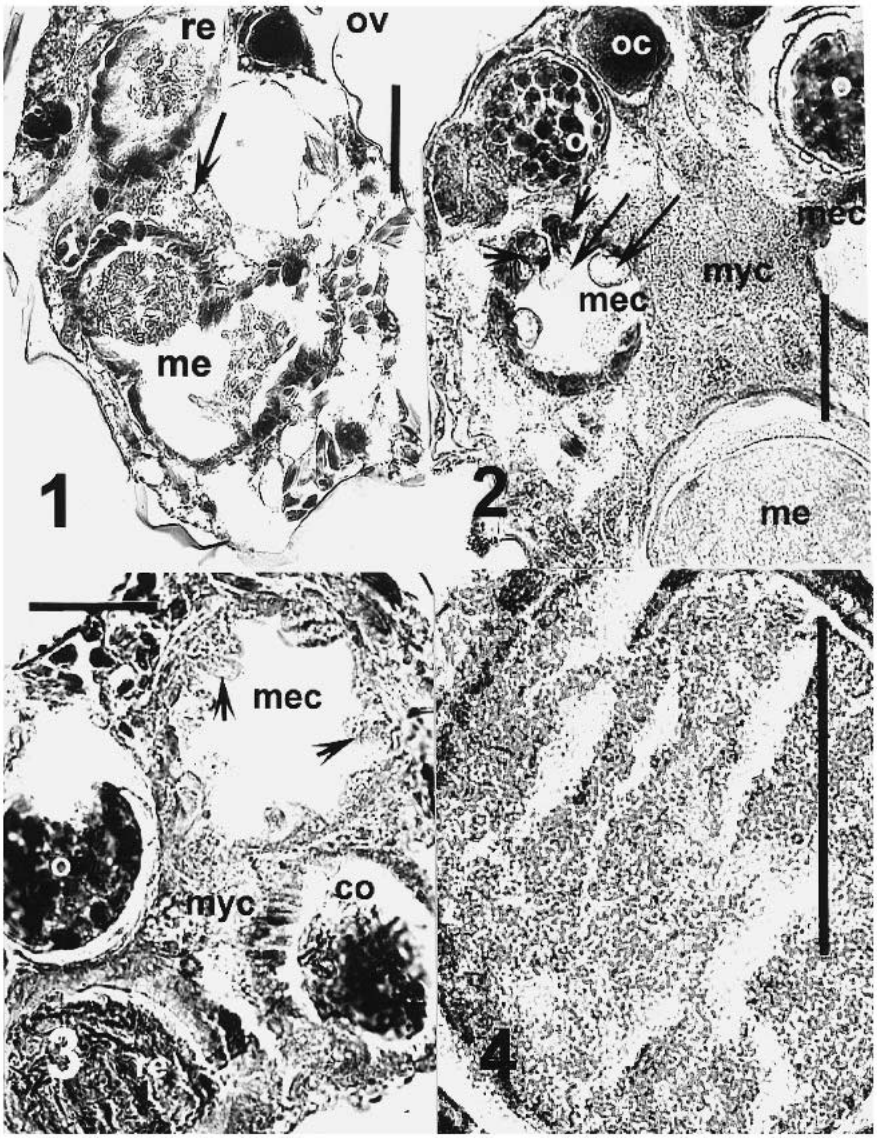
All results were synchronous, without qualitative variability among replicates in each experimental group tested by the methodology used (histology, plating, enzymes), just as in previous experiments with mites of this species (cf. Smrž 1991, 1996, 2000; Smrž and Čatská 1989, 1991; Smrž and Trelová 1995).

Results

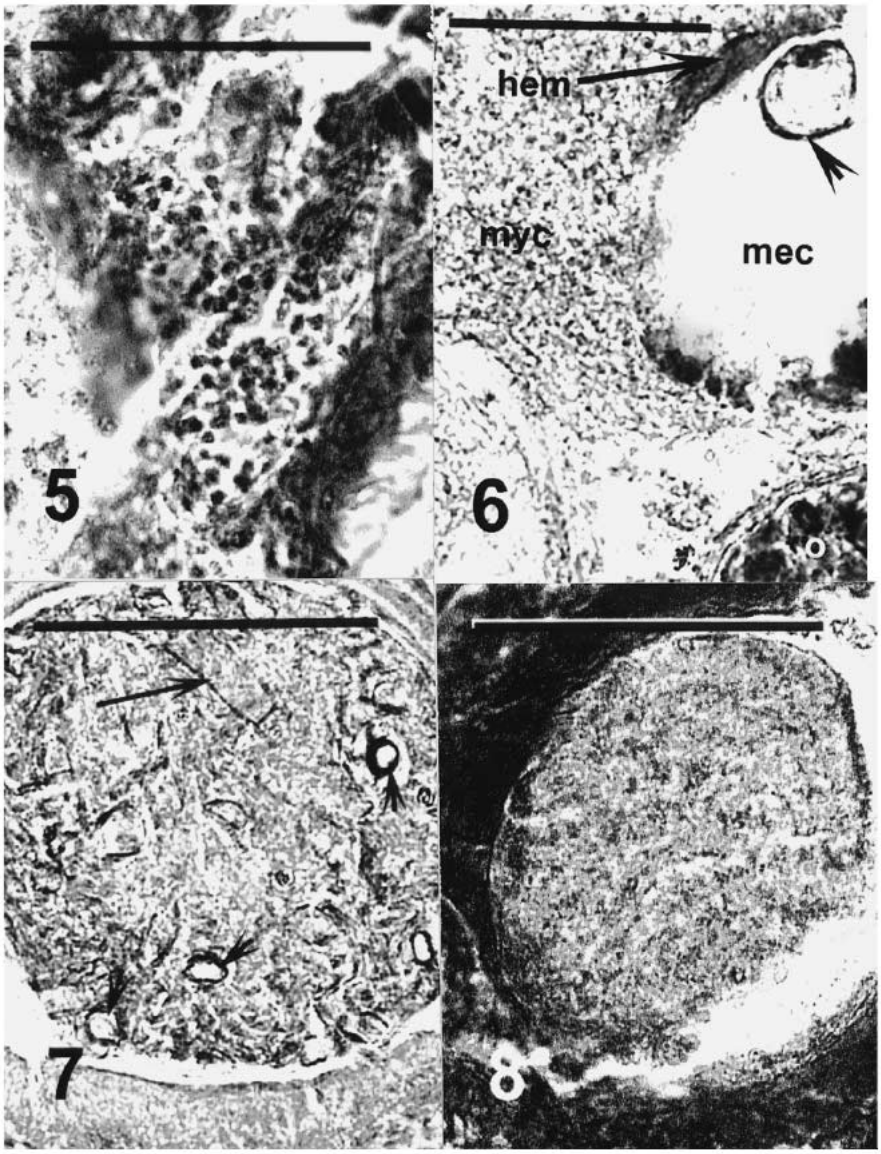
On *Alternaria*, the food boli in the mesenteron consisted of rough fragments of fungal hyphae and spores throughout the alimentary tract (Figures 1, 3 and 7). The bacteria were dispersed throughout the body cavity between the mesenchymal cells outside the gut. They did not form compact bodies (Figures 5 and 10). The mites contained only small groups of scarce, but large, bacterial cells. The TEM confirmed their rather large size and revealed their morphological heterogeneity (Figure 10). Chitinase activity was not detected. *Alcaligenes faecalis* strongly dominated (over 20 CFU per dish) among the bacteria plated from the mite homogenate. Only 1–2 CFU of *Agrobacterium* sp. occurred per dish.

On *Penicillium*, the food boli consisted of very fine granules and fragments mixed with mucus (Figures 2, 4 and 8). The bacteria formed bodies adjoining the alimentary tract especially between the mesenteron and mesenteral caeca (Figures 2 and 6). The bacteria were closely adjacent to each other, their cells were small. Under the TEM, the morphological heterogeneity of bacteria inside these bodies was minor (Figures 11 and 12) in comparison with the *Alternaria* experiment. Chitinase activity was detected. *Serratia marcescens* dominated (over 10 CFU per dish) among bacteria isolated from mites, accompanied by 1–2 CFU of *Achromobacter* sp. per dish.

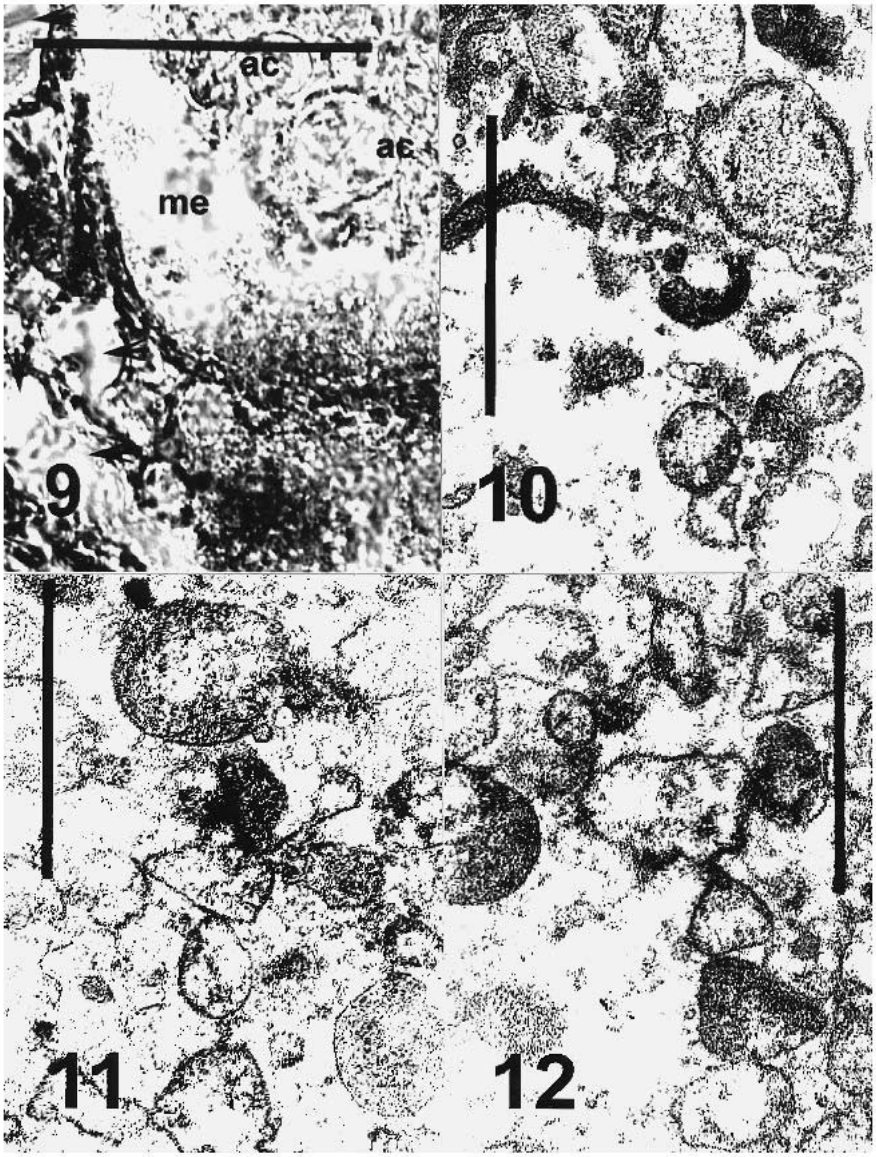
On the algal food, the algal cells dominated completely in the mite gut after one day, whereas after three days hyphal fragments and spores of fungi invading the algal cover appeared within the gut. The bacterial population consisted of only a few cells in the mesenchymal tissue in one histological section during three days at the start of experiment. Their number increased after three days but, again, no conspicuous cell number (less than 10 cells in one histological section) was recorded (Figure 9).



Figures 1– 4. Tyrophagus putrescentiae, the alimentary tract: 1) the food boli in mesenteron and rectum of mites grazing on *Alternaria,* arrow points to the group of bacteria, parasagittal section. 2) the group of bacteria of a mite grazing on *Penicillium*, arrows point to the apocrine secretion in mesenteral caeca, horizontal section, arrowheads point to hemocytes. 3) a detailed view of the alimentariy tract of a mite grazing on *Alternaria*, arrowheads point to the apocrine secretion, parasagittal section. 4) mesenteron of a mite grazing on *Penicillium* containing mainly bacterial cells in amorphous substance, parasagittal section. Masson's triple stain. Scales: 20 μm. Abbreviations used: co – colon, me – mesenteron, mec – mesenteral caecum, myc – group of bacteria, o – egg, oc – oocyte, ov – ovarium with oocyte, re – rectum.



Figures 5–8. Tyrophagus putrescentiae, internal group of bacteria and faecal pellets: 5) detail of the internal group of bacteria body of a mite grazing on *Alternaria*, parasagittal section. 6) detail of the group of bacteria of a mite grazing on *Penicillium*, arrowhead points to the apocrine secretion. 7) faecal pellet in the rectum of a mite grazing on *Alternaria*, arrow points to a fragment of fungal hypha, arrowheads point to the fungal spores. 8) faecal pellet containing the bacterial cells (small dark points) in the amorphous substance in the rectum of a mite grazing on *Penicillium*. All parasagittal sections. Masson's triple stain. Scales: 20 μm. Abbreviations used: hem – hemocyte in the caecal wall, mec – mesenteral caecum, myc – group of bacteria, o – egg.



Figures 9–12. *Tyrophagus putrescentiae*, internal group of bacteria: 9) mesenchymal tissue around the mesenteron of a mite grazing on the algae, arrowheads point to the empty mesenchyma cavities. 10) detail of the group of bacteria in a mite grazing on *Alternaria*. 11, 12) details of the group of bacteria in a mite grazing on *Penicillium*. All parasagittal sections. Masson's triple stain (9), TEM (10–12). Scales: 20 μm (9), 3 μm (10–12). Abbreviations used: ac – algal cells, me – mesenteron.

Discussion

The presence of internal extra-intestinal bacteria was correlated with the type of food. As reported in the literature, their growth can be induced by mycophagy as supported by the microanatomy of the alimentary tract (*Damaeus*: Smrž 1996; Smrž and Trelová 1995; *Chamobates voigtsi:* Černý 1991, 1999; *Tyrophagus putrescentiae*: Smrž and Čatská 1989). Food boli consisted of hyphal fragments and spores (*Damaeus* and *Chamobates*, from the field samples of Smrž and Trelová 1995 and Černý 1999, respectively) or fine granulation (*Tyrophagus* from the experiment). The pure algal food did not induce this phenomenon.

As published in our previous papers (Smrž and Čatská 1987, 1989; Smrž and Trelová 1995; Smrž et al. 1991), the occurrence of *Serratia* was not detected on the sterile fungal colonies on malt agar before the start of experiments. Their dominance inside the mite body, if any, increased during the consumption of some fungi (especially some species, or their strains, of *Penicillium*).

The species, maybe strains, of fungi seem to be important in this respect (Smrž and Čatská 1989). The pattern of the digestion of different fungi was correlated with different types of food boli as well as different development of the internal bacterial bodies between the mesenchymal cells between the internal organs.

In these experiments, *Penicillium* was grazed in a different pattern from *Alternaria*. The cell content appeared to be drained and propagula finely crushed in *Penicillium*, whereas *Alternaria* hyphae and some of the spores were roughly crushed or intact. The chitinolytic activity or presence of bacteria (*Serratia marcescens*; see also Smrž and Čatská 1989; Smrž and Trelová 1995; Smrž 1996, 1998, 2000) were not recorded on *Alternaria* in contrast to the other fungus finely crushed by grazing of mites. One can assume the digestion of cell walls of *Penicillium* in addition to the cell contents.

In conclusion, the food type, here fungal species (in this experiments *Penicillium*), and pattern of their grazing and digestion is important for the association of mites and internal, extra-intestinal bacteria. The fine crushing of the fungal cell walls yields suitable a substrate for nitrogen release: chitin. The populations of chitinolytic bacteria (*Serratia*) increase between the internal mite organs up to the forming of defined groups accompanied by chitinase activity. Other food did not induce such associations in this experiment.

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