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A new strategy of endosymbiont midgut bacteria in ant (Ponerinae)

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ABSTRACT

The location and ultrastructure of bacteria associated with microvilli in the midgut of *Odontomachus bauri* were examined by transmission electron microscopy. These filamentous type bacteria are the second morphotype described in the midgut of this ant. They colonizes only the ectoperitrophic space, more specifically attaching along microvilli. A thick capsule attaches bacteria to microvilli and protect them from acidic pH and digestive enzymes. Details of the location and association with microvilli are discussed.

1. Introduction

Associations of microorganisms with the digestive tract of insects are varied and widespread. Reports of insect–microorganisms association usually describe bacterial population in the gastric caeca, hindgut or ileum (Bignell, 1983; Bution and Caetano, 2008; Bution et al., 2007, 2008; Caetano, 1984, 1988; Caetano and Cruz-Landim, 1985; Roche and Wheeler, 1997, Wigglesworth, 1974).

Bacterial colonization of the midgut epithelium is less common and frequently associated with insects that lack a peritrophic membrane (Bignell et al., 1980a,b).

The peritrophic membrane of insects is basically a chitinous sheath that surrounds the food bolus from the previous region and compartmentalizes the midgut in endo and ectoperitrophic spaces (Terra, 2001; Terra and Ferreira, 1994; Terra et al., 1996).

In addition to lining and protecting the midgut against injuries (mechanical or chemical) that may occur during the passage of the food bolus, the peritrophic membrane is also thought to prevent the colonization of the midgut epithelium by microorganisms (Lehane and Billingsley, 1996; Terra, 2001; Terra et al., 1996). However, ultramorphological studies have reported the presence of bacteria associated with ectoperitrophic space of the midgut of *Procubiterines abitirienisis*, *Reticiuliterines flavipes*, *Coptoterinesforinosanius* (Isoptera), *Acheta domestica* (*Orthoptera*) and in larvae of *Xylotopus par* (*Diptera*). Bacteria were observed free, attached to the epithelium surface or between the peritrophic membrane (Bignell et al., 1980a,b; Ulrich et al., 1981; Bignell, 1983; Caetano, 1984; Kaufman et al., 1986; Caetano, 1988; Caetano and Rodrigues, 2001).

The presence of bacteria is uncommon in the midgut of insects also due to the enzymes present in this region that could prevent their survival. However, symbiotic bacteria have been described in the midgut of several ant species, such as those of the genus *Camponotus* (Blochmann, 1882; Schroder et al., 1996). These bacteria are present inside specialized cells in the midgut termed "bacteriocytes".

In the midgut of *Odontomachus bauri*, Caetano et al. (2008) described the presence of bacteria termed "endocytobionts" inside epithelial cells. These authors suggest the participation of these bacteria in the digestion of food, thus acting as symbiotic organisms that might open new possibilities to exploit food resources in the environment.

Bacteria have also been reported in the midgut of *Dolichoderus* (=*Monacis*) *bispinosus*, *D.* (=*M.*) *attelaboides*, and ants of the tribe Cephalotini (Caetano and Cruz-Landim, 1985; Caetano, 1989, 1990; Caetano et al., 1989, 2002; Roche and Wheeler, 1997; Bution et al., 2006; Bution and Caetano, 2008).

Although bacteria have already been described in the midgut of *O. bauri*, filamentous type bacteria were later found attached to microvilli of columnar cells. Thus, in this study, the ultramorphology of the apical region of columnar cells of the midgut of *O. bauri* were examined to describe the association of these bacteria with microvilli.

2. Materials and methods

Specimens of *O. bauri* were collected in Rio Claro city, São Paulo state, Brazil. The midgut was carefully removed from the abdomen

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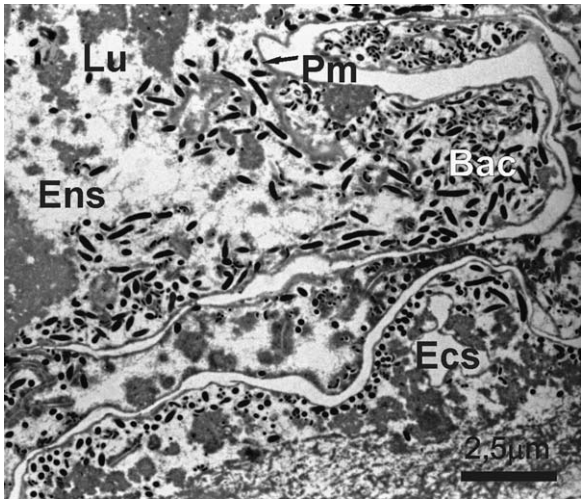


Fig. 1. Electron micrograph of the midgut of *Odontomachus bauri* showing bacteria present in the endoperitrophic space (Ens) attached to the peritrophic membrane (Pm) and in the ectoperitrophic space (Ecs). Bacteria (Bac), lumen (Lu). Scale bar.

directly in cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and fixed for 2 h. Postfixation was carried out in 1% osmium tetroxide in the same buffer. En bloc staining with 2% uranyl acetate was followed by a series of ethanol dehydration steps.

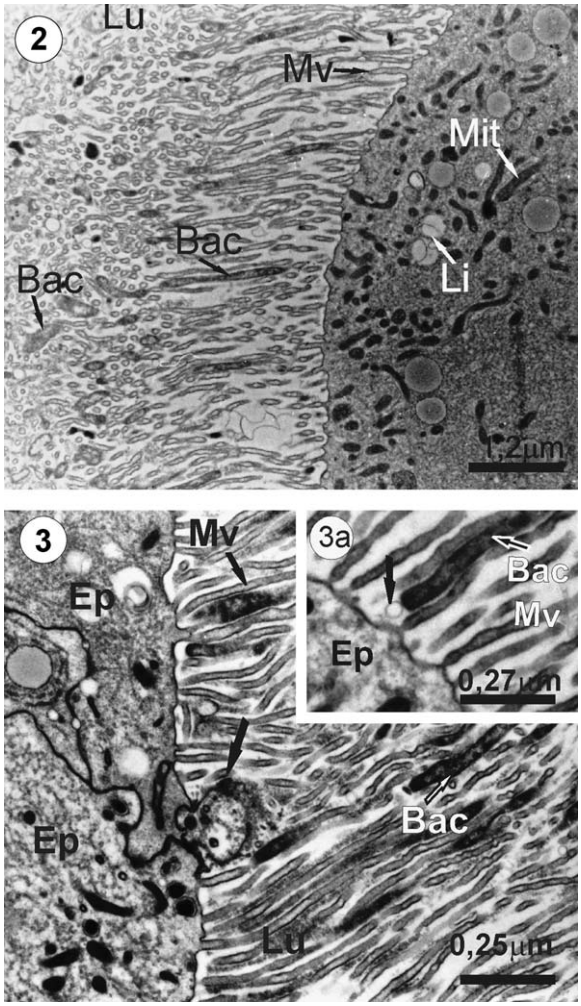
Midgut were then embedded in Epon-Araldite resin and sectioned with a Leica ultramicrotome. Ultra-thin sections were stained with uranyl acetate and lead citrate and photographed with a CM 100 Philips TEM at 80 kV.

3. Results and discussion

The ultrastructural analysis of sections of the anterior portion of the midgut of *Odontomachus bauri* revealed the presence of bacteria inside epithelial cells, termed "endocytobionts", between the peritrophic membrane and the endoperitrophic space (Caetano et al., 2009) (Figs. 1 and 2). Although these were the first bacteria described inside the midgut cells of this ant, other filamentous bacteria were later observed attached to microvilli of columnar cells (Figs. 2, 3a and 4).

In the ant's midgut of the Cephalotini tribe and of the *D. attelaboides* and *D.* (=*M*) *bispinosus*, symbiotic bacteria were described in the ectoperitrophic space (Caetano, 1984, 1988, 1989; Roche and Wheeler, 1997; Bution and Caetano, 2008). In Cephalotini tribe, the bacteria type is coccus and they can be found in the lumen and between the microvilli, different from the observed in the present study, where the filamentous bacteria are arranged parallel to the microvilli and never free in the lumen. The filamentous bacteria promote a great interaction with the microvilli when they assume them disposition. Despite differences among morphotypes, the strategy of bacterial colonization between microvilli is in general similar, but the filamentous showed more specific because they are arranged in narrow contact with the apical cell surface. This strategy provides protection to bacteria because they do not stay along the food way, in addition they have a direct contact with the material released by digestive cells. This interaction is also supported by the glycocalyx, as showed in many figures. In the present study, the new strategy concern to the attachment of these bacteria along columnar cells microvilli, increasing the surface of contact area.

Our findings also suggest that the exchange of compounds between the midgut epithelium and bacteria might occur through the attachment site of bacterial capsules and the glycocalyx



Figs. 2 and 3. Electron micrograph showing details of the apical region of columnar cells of the midgut of *O. bauri*. Note vesicles being released through the apical region of the cell (arrow) as well as microvilli (Mv). Lipid droplets (Li).

(Figs. 2, 3a and 4), as observed in the biofilm formed by colonies of enteric bacteria in rats, baboons, and humans (Palestrant et al., 2004).

The thick bacterial capsule clearly play a role in protecting bacteria against chemical and physical injuries caused by the action of digestive enzymes and peristaltic movements.

An increasing number of studies describing the presence of bacteria in the ectoperitrophic space of the midgut of insects has been conducted and contributed to understand the characteristics of this relationship (Bignell, 1983; Bignell et al., 1980a,b; Bution and Caetano, 2008; Caetano, 1988, 1989; Roche and Wheeler, 1997). Ultrastructural studies have demonstrated symbiotic relationships involving the presence of bacteria in the ectoperitrophic space of the midgut of *Procubiterines abitirienisis*, *Reticuliterines flavipes*, *Coptoterines forinosanius* and in larvae of *Xylotopus par* (Diptera: Chironomidae). According to these authors, these bacteria are attached to microvilli as well as free in the ectoperitrophic space, as observed in *O. bauri*. However, the shape of bacteria was different to the one described in our study in all the cases described, as most were cocci, and none of them were filamentous type bacteria.

Some authors have suggested that the peritrophic membrane may prevent the colonization of the midgut epithelium by microorganisms (Terra, 2001; Terra et al., 1996). However, in *O. bauri* and other ants, bacteria have been observed in the