

MICROORGANISMS ASSOCIATED TO THE EPITHELIUM OF THE DIGESTIVE TRACT OF *ENANTIODRILUS BORELLII* COGNETTI, 1902 (ANNELIDA: GLOSSOSCOLECIDAE)

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[Picón, M. C., Teisaire, E. S., Nieto, O. L. & Albarracín, V. 2019. Microorganisms associated to the epithelium of the digestive tract of *Enantiodrilus borellii* Cognetti, 1902 (Annelida: Glossoscolecidae). Munis Entomology & Zoology, 14 (1): 165-169]

ABSTRACT: Earthworms (Annelida, Oligochaeta) essentially contribute to terrestrial ecosystems, modifying soil's physical and chemical properties with the presence of bacterial microflora in the digestive tract. Bacteria associated to the intestinal epithelium and the adhesion forms that allow their presence in the digestive tract are identified through the techniques with scanning electron microscopy. Results show microorganisms that were not dragged by washing in the fixation technique used. Adhered colonies are observed in the anterior part of the intestine, at calciferous glands level; whereas they are not observed in the posterior part of the intestine, at typholosome level. The morphology of strains is cocci, streptococci and bacilli, constituting colonies tangled among cilia of the digestive epithelium cells. Individual fixation structures are not visualized, being mucous secretion the way of aggregate adhesion.

KEY WORDS: Annelida, digestive tract, microorganisms, epithelium

Earthworms (Annelida, Oligochaeta) play an important role in terrestrial ecosystems, contributing to soil fertility by modifying its physical, chemical and biological properties (Edwards & Lofty, 1972; Syers & Springett, 1984; Krishnamoorthy, 1985; Hoogertkamp et al., 1983; Lavelle, 1983; Lavelle & Martin, 1992; Egert et al., 2004). The actions of earthworms are performed partly by the presence of a diverse bacterial microflora in the digestive tract (Lee, 1985; Edwards & Fletcher, 1988; Vinotha et al., 2000; Fukatsu & Nikot, 1998; Santiago, 1995; Alonzo et al., 1999). Toyota & Kimura (2000) and Idowu et al. (2006) have studied intestinal microorganism communities, indentifying those that are characteristic or endogenous of each species of earthworm. Through scanning electron microscopy, cocci, bacilli and filamentous microorganisms were found on intestinal walls of *Lumbricus terrestris* and *Octolasion cyaneum* (Jolly et al., 1993) and *Onychochaeta borincana* (Mendez et al., 2003; Valle-Molinares et al., 2007).

Enantiodrilus borellii Cognetti, 1902 is a Glossoscolecidae distributed in Argentina, Bolivia and Brazil. In previous research on the bacterial flora of *E. borellii*, an endogenous microflora of this species was identified (*Oscillospira* spp. and *Bacillus* sp.), as well as microorganisms ingested of the soils where they inhabit (Picón & Teisaire, 2012; Picón et al., 2014). This is the reason why, through the use of scanning electron microscope techniques, it is expected to find bacteria associated to the intestinal epithelium and adhesion forms that facilitate its permanence inside of the digestive tract.

MATERIALS AND METHODS

Earthworms were collected on May 2012, in a sugar cane farm, in Chicligasta, Tucumán, Argentina (S 27°29'26" – W 65°37'18"). Samples were identified according to the described characteristics published by Moreno et al. (2005). They were collected and processed according to Picón et al. *op cit.* technique. Samples were set in formadehyde 4%, for a minimum of 24 hours. Two parts of the intestine were extracted with dissection: the anterior part at calciferous glands level and the posterior part at typhlosole level. The content was extracted by soft wash with a fixative using Pasteur pipette and under magnifying glass. Dehydration was done with a series of ethanol (30% - 100%) and acetone 100%, for further drying at critical point, coated with gold and mounting of samples. The observations were done under scanning electron microscope Zeiss Supra 55VP. Photomicrographs of the anterior and posterior part of the digestive tube were taken.

RESULTS

Observations of the digestive epithelium surface reveal the microorganisms which have been attached to the surface of the epithelium, even after the washing in the fixative liquid. A significant difference was distinguished in terms of the anterior and posterior parts of the dissected digestive tube. Aggregate colonies were observed in the anterior part, whereas they weren't in the posterior one. The morphology of the microorganisms found was cocci (Figs. 1, 2), streptococci (Fig. 3) and bacilli (Fig. 4), constituting, in all the cases, colonies added to each other and tangled with the long cilia of the digestive epithelium cells. Individual fixation structures or elements were not observed and the colonies form a unit cemented by mucous and adhered to the epithelium surface.

DISCUSSION

In coincidence with other authors' results (Menendez et al., 2003; Jolly et al., 1993) it has been observed that most of the microflora is found in the anterior part of the intestine, however, unlike them we did not find microorganisms in the posterior part. This outcome might be related to the washing technique used and the previous preparation of the digestive tube for the observation under the scanning electron microscope. This analysis coincides with the findings of Valle-Molinares et al. (2007) whose opinion is that only few microorganisms resist the intense washing procedure of the intestine, as consequence of the employed technique; which allow the recognition of a strong attachment of the organisms to the intestinal walls. This microorganism-digestive tube relationship makes it possible the identification of endogenous species with a significant biological function in these earthworms.

Employing techniques similar to the ones we used in this study, Menendez et al. (2003) found filamentous bacteria adhered to the intestinal walls by a structure they called "socket-like". Nevertheless, in our studies fastening structures were not found; we only observed colonies adhered to each other and tangled in the cilia of the digestive wall.

In *Enantiodrillus borellii* three morphological types of microorganism could be distinguished: cocci, streptococci and bacilli; on the contrary Jolly et al. (1993) observed the presence of cocci, some filaments and few bacilli in *Lumbricus terrestris* and *Octolasion cyaneum*. While, it was identified in *Onychochaeta*

borincana filamentous bacteria and spores (Menendez *op cit.*) and lined bacilli (Valle-Molinares et al. *op cit.*). These results would confirm the great species diversity of the intestinal microflora in earthworms, in reference to their morphology and functional behavior.

The morphological types found, cocci and bacilli, among other tests, helped to identify the bacterial strains found in *Enantiodrilus borellii* in previous studies; in which the strains of the digestive tube, not present in the soil, were indicated as endogenous: *Oscillospira* sp., *Bacillus* sp., *Azomonas* sp. and *Acidiphilium* sp. (Picón & Teisaire, 2012). At the same time, with a recolonization technique of the sterile soil *Oscillospira* sp. and *Bacillus* sp. could be reconfirmed as endogenous (Picón et al., 2014). In this respect, we highlight as an important fact that both strains are presented with a cocci and bacilli morphology respectively, in coincidence with the results in the scanning electron microscope observations.

ACKNOWLEDGEMENTS

Thanks to the technicians of Centro Integral de Microscopía Electrónica (CIME), especially to Mr. Luciano Martinez and Lic. Natalia Leyria. Tec. Pablo Pereyra, Fundación Miguel Lillo.

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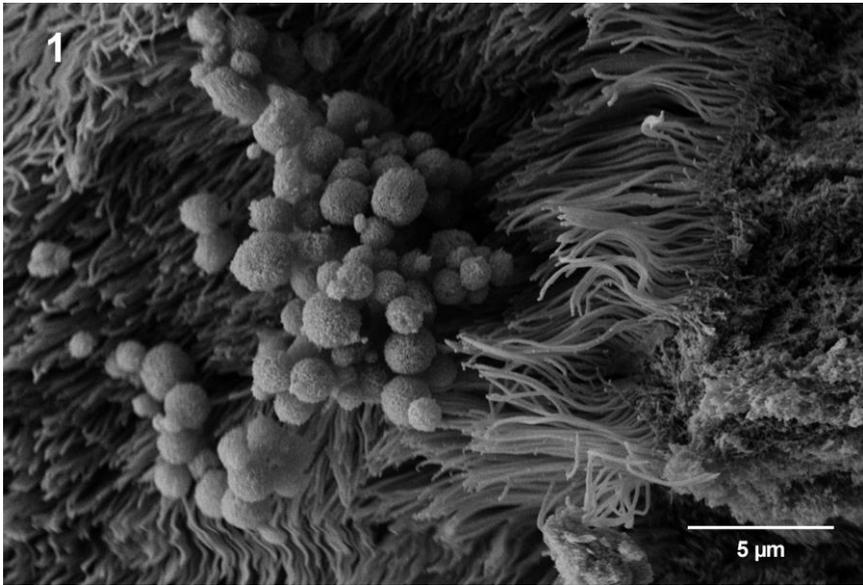


Figure 1. Section of the anterior intestine: cocci aggregate in mass on the intestinal ciliated epithelium.

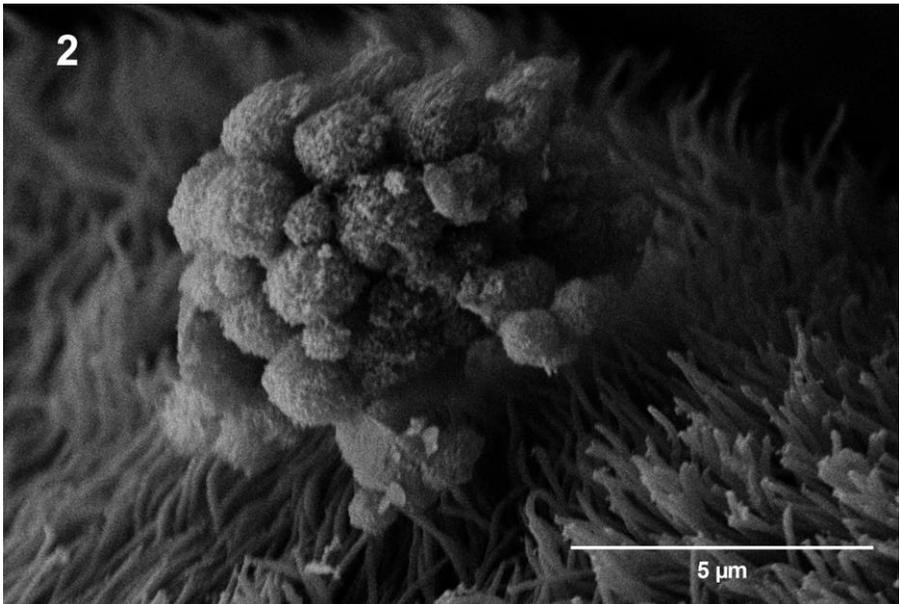


Figure 2. Section of anterior intestine: cocci aggregate on the intestinal ciliated epithelium.

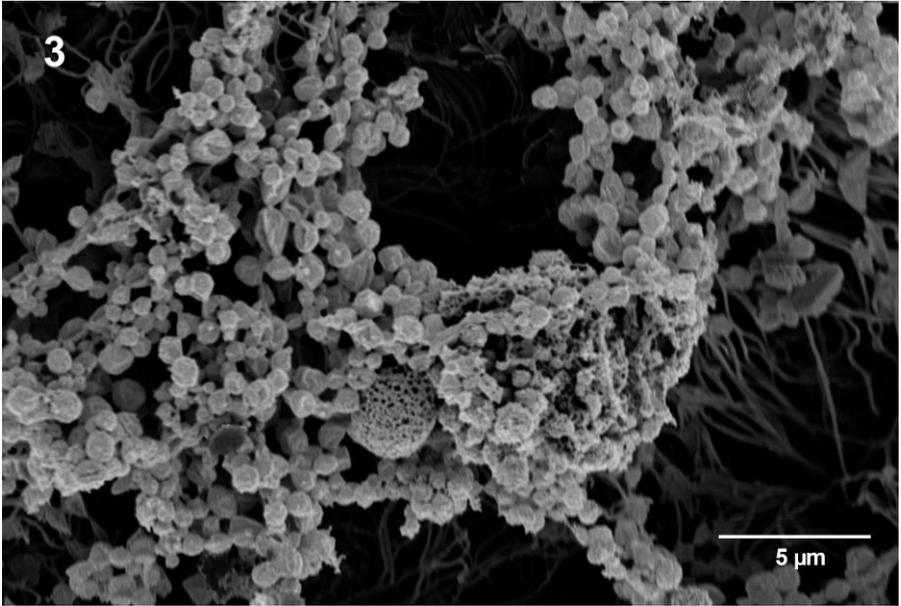


Figure 3. Section of anterior intestine: streptococci on the intestinal ciliated epithelium.

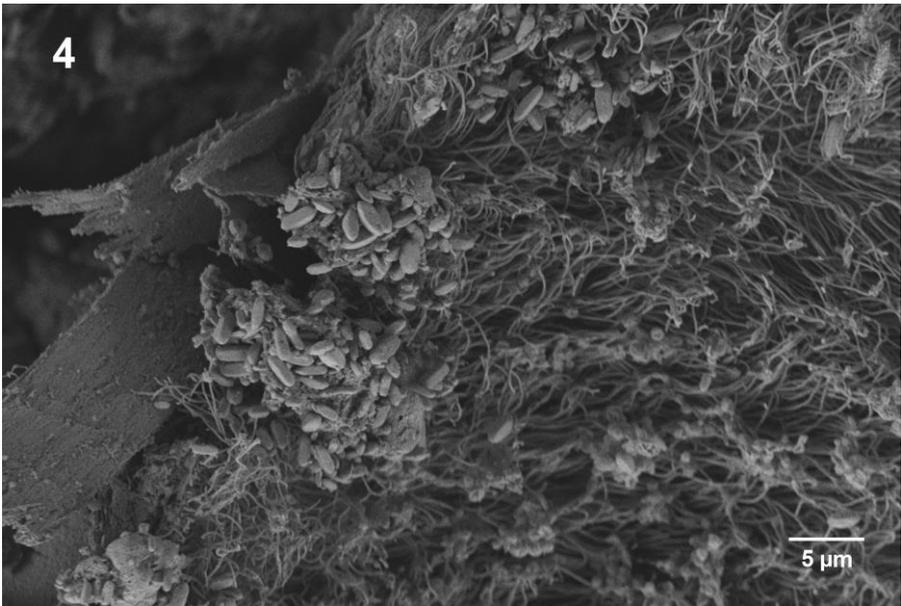


Figure 4. Section of anterior intestine: bacilli colonia on the intestinal ciliated epithelium.