

Vicente et al., Natural bacterial communities associated with the pine sawyer beetle *Monochamus galloprovincialis*. In: Schröder, T. (ed.), Pine Wilt Disease Conference 2013, pp. 63-64, Braunschweig, ISSN: 1866-590X

Natural bacterial communities associated with the pine sawyer beetle *Monochamus galloprovincialis*

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Most studies of cerambycids microbiota are related with gut-bacterial communities due to their importance in the insect's biology and ecology. Essentially, the research in this field showed that insect microbial communities are limited by specific niche characteristics. The present study investigates the natural bacterial communities of *Monochamus galloprovincialis* collected from Portuguese *Pinus pinaster* trees and *Bursaphelenchus xylophilus*-free, using a metagenomics approach. A total of 492 partial sequences (750-1200bp) of 16S rRNA gene were considered in this study. The rarefaction curves analyses showed 18 OTUs (operational taxonomic units) at genus-species level (95-97% sequence similarity). Bacterial communities associated with *M. galloprovincialis* are mainly composed by Proteobacteria (78.5%), followed by Firmicutes (20.8%) and Bacteroidetes (<1%). From the phylum Proteobacteria, three classes were present: γ -proteobacteria (87.9%), β -proteobacteria (11.6%), and α -proteobacteria (0.5%). The most abundant genera of Proteobacteria were *Serratia* (76.4%), followed by *Janthinobacterium* (11.6%), *Rahnella* (5.0%), *Pseudomonas* (3.6%), and *Nevskia* (2.1%). Among Firmicutes, the genera found were: *Bacillus* (95%), *Paenibacillus* (3%), *Lactococcus* (1%), and *Lysinibacillus* (1%). From the phylum Bacteroidetes, representatives were identified as *Sphingobacterium* (n=1), *Sediminibacterium* (n=1) and uncultured *Bacteroidetes* (n=2). Our results show a predominance of γ -proteobacteria in *M. galloprovincialis*, which might be intrinsically related with their feeding diet and habitat characteristics. Interestling, a high-density population of *Serratia* spp. was found in *M. galloprovincialis*. The presence of *Serratia* in insects is well documented, both as symbiont or pathogen, and its related with their fitness ability to resist antibacterial substances ingested by the insect, as well as the powerful enzymatic cocktail produced (chitinases, lecithinases, and proteinases) (Grimmont and Grimmont, 2006). Previous culture-dependent studies have also reported isolation of *Serratia marcescens* in *M. alternatus* (Ma et al., 2009),

Aerobacter aerogenes and *Bacillus cereus* var. *mycoides* in *M. scutellatus*, *M. notatus* and *M. marmorator* (Soper and Olsen, 1963). Further studies are needed to understand their functional contribution to the bacterial community structure of *M. galloprovincialis*. In light of all knowledge regarding bacterial communities of *B. xylophilus* and the results here presented, it is tempting to establish the hypothesis that perhaps the *B. xylophilus* can harbour bacteria from the insect. An example is the predominance of *Serratia* in *M. galloprovincialis* and also *B. xylophilus* (Vicente *et al.*, 2011). Vicente *et al.* (2012) described that some nematode-associated bacteria, including *Serratia* spp., were able to degrade cellulose, an advantage in the adaptation and colonization of wood tissues (Harakava and Gabriel, 2003). Although with this study it's not possible to establish a comparison between insect-vector and *B. xylophilus* bacterial communities, the results presented are useful and encourage future work in this subject (Vicente *et al.*, 2013). Understanding the role of bacteria transmission in the PWD complex will bring important knowledge for future prospects in the disease management and control.

REFERENCES

- Grimmont F; Grimmer PAD (2006). The Genus *Serratia*. In: *The Prokaryotes - a Handbook on the biology of Bacteria: Ecophysiology, isolation, identification, Application*. New York, NY: Springer Verlag. 219–244.
- Harakava R; Gabriel DW (2003). Genetic differences between two strains of *Xylella fastidiosa* revealed by suppression subtractive hybridization. *Applied Environmental Microbiology* 69: 1315–1319.
- Ma LJ; Zhang LQ; Lin HP; Mao SQ (2009). Investigation of pathogens of *Monochamus alternatus* in East China and virulence. *Chinese Journal of Biology Control*, 25:220-224.
- Soper RS; Olsen RE (1963) Survey of biota associated with *Monochamus* in Maine. *Canadian Entomology* 95: 83-95
- Vicente CSL, Nascimento F, Espada M, Mota M & Oliveira S (2011) Bacteria associated with the pinewood nematode *Bursaphelenchus xylophilus* collected in Portugal. *Antonie Van Leeuwenhoek Journal of Microbiology* 100: 477–81.
- Vicente CSL; Nascimento FX; Espada M *et al.* (2012) Characterization of bacteria associated with pinewood nematode *Bursaphelenchus xylophilus*. *PloS one* 7: e46661.
- Vicente CSL; Nascimento FX; Espada M; Barbosa P; Hasegawa K; Mota M; Oliveira S (2013). Characterization of bacterial communities associated with the pine sawyer beetle *Monochamus galloprovincialis*, the insect vector of the pinewood nematode *Bursaphelenchus xylophilus*. *FEMS Microbiology Letters* (accepted).