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Diversity and *in vitro* nematocidal activity of bacteria associated to pinewood nematode

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Bacteria have been suggested to play a role on pine wilt disease since they have been isolated associated with the pinewood nematode. The aim of this work was to evaluate the diversity of the nematode associated bacteria and their potential role in this disease by determining their in vitro nematocidal activity. The bacterial isolates, identified by 16S rRNA gene sequence, belonged to the families Microbacteriaceae, Oxalobacteriaceae, Burkholderiaceae, Enterobacteriaceae, Pseudomonadaceae and Xanthomonadaceae. The most nematocidal strain, *Serratia* sp. A88copa13, produced proteases in the supernatant.

INTRODUCTION

Bacteria have been suggested to play a role on pine wilt disease (PWD) since they have been found associated with the pinewood nematode (PWN), *Bursaphelenchus xylophilus*. Therefore, PWN isolates from across the globe have been studied in order to understand whether these bacteria can produce toxins that could be involved in the development of PWD (Proença et al. 2010). The microbial community associated to PWN was accessed in nematodes from different recently affected areas in Portugal. The aim of this work was to evaluate the diversity of the nematode associated bacteria and their potential role in the PWD by determining their in vitro nematocidal activity.

MATERIALS AND METHODS

The microbial community associated to PWN was assessed isolating the strains on the track of nematodes from infected *Pinus pinaster* trees, from affected areas in Portugal. The bacterial isolates were identified by 16S rRNA gene sequence. Phylogenetic analysis were performed by using ARB software package and type strains from international databases. All isolates were screened for their ability to produce siderophores, lipases and

proteases (Proença *et al.* 2010). Strains were tested against *B. xylophilus* to evaluate their nematicidal activity assessed as the percentage of dead nematodes when incubated with bacteria supernatant during 24 h at 26°C.

RESULTS

Strains isolated belonged to the families *Microbacteriaceae*, *Oxalobacteriaceae*, *Burkholderiaceae*, *Enterobacteriaceae*, *Pseudomonadaceae* and *Xanthomonadaceae*. Forty-seven strains were tested and 21 strains produced extracellular products with nematicidal activity (Figure 1). The most nematicidal strain, *Serratia* sp. A88copa13, produced proteases in the supernatant. Biological assays revealed differences in nematicidal activity of the proteases to different species of *Bursaphelenchus*.

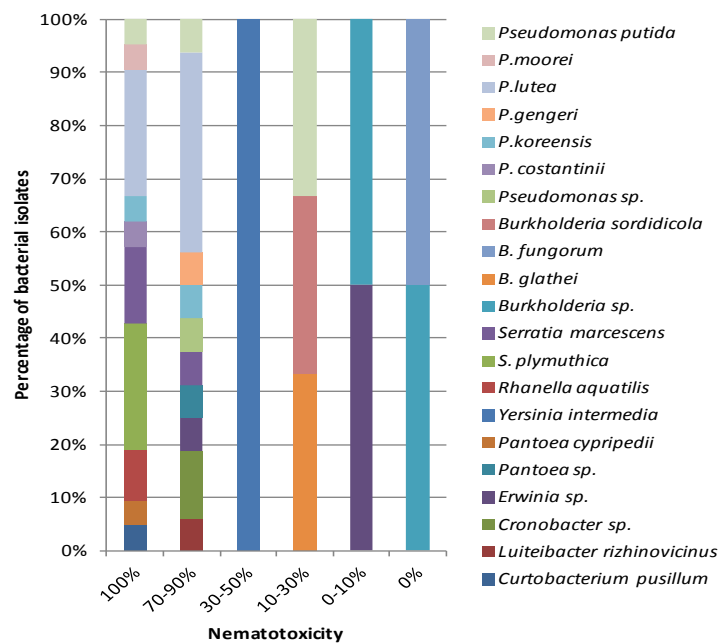


Figure 1 — Biochemical properties and nematicidal ability of bacteria associated with *Bursaphelenchus xylophilus*. Most strains produced siderophores and lipases.

CONCLUSIONS

In Portugal, strains belonging to the families *Enterobacteriaceae* and *Pseudomonadaceae* have been isolated associated to the PWN and some have potential to eliminate the nematode *in vitro*. In this process, proteolytic enzymes and lipases, surfactants and possibly siderophore, produced by the bacteria to the extracellular medium, may be involved, being the proteases (metalloproteinases and serine) the most relevant.

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