

# Isolation and genetic characterization of bacteria associated with *Philaenus spumarius* for the control of *Xylella fastidiosa*

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**Abstract:** The endosymbiotic bacteria that live within the body of insects are involved in many aspects of the host physiology, including reproduction and defense. Thus, the exploitation of these microorganisms may have practical applications for the management of vector borne diseases. In the Mediterranean area, *Philaenus spumarius* was identified as the main vector of *Xylella fastidiosa*. This xylem-restricted bacterium is responsible for several diseases in a variety of agricultural crops of high importance, for which there aren't any effective control method. Thus, in this work we evaluate different media types for the isolation and growth of bacteria living within *P. spumarius* adults, for their potential exploitation in the management *X. fastidiosa*. Specifically, was compared the effect of minimal (Luria Bertani - LB) and complex (Modified Melin-Norkrans - MMN) media, with and without fetal bovine or gelling agents, on the number and diversity of bacteria. The collection of isolates obtained was further genetic characterized by BOX-PCR and sequencing of the 16S ribosomal RNA (rRNA) gene. Results showed no significant differences on the abundance and diversity of bacteria among the two media tested (LB and MMN). The addition of fetal bovine to the media leads to a slight increase in bacterial abundance, in particular in MMN medium. While the liquid media lead a significant increase in abundance, the solid media facilitated the growth of more diverse bacterial taxa. The comparison between BOX-PCR and 16S rRNA gene sequencing for the analysis of 57 bacterial isolates, revealed a greater discriminatory power of the former, allowing the differentiation of the bacteria even at the intra-species level. Clustering of the isolates using BOX-PCR fingerprinting was different to that obtained from the 16S rRNA gene phylogenetic tree. 16S rRNA gene sequencing method proved to be more suitable in phylogenetic evaluations, generally grouping isolates belonging to the same genus..

**Keywords:** Culture media; DNA fingerprinting; BOX-PCR; 16S rRNA gene sequencing

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