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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. *Philaenus spumarius* was identified as one of the main vector of *Xylella fastidiosa*, a bacterium responsible for several diseases in a variety of agricultural crops of high importance. In this work, different media types were evaluated for the isolation 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 abundance and diversity of bacteria. The collection of isolates obtained and of others previously obtained was further characterized by BOX-PCR and sequencing of the 16S ribosomal RNA (rRNA) gene.

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

Philaenus spumarius

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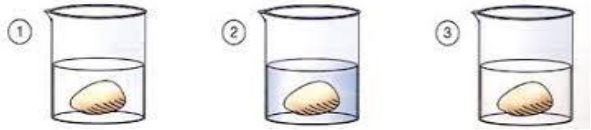
Material and Methods

Sampling

Bragança (Portugal)

Isolation of bacteria

Surface sterilization of adults



1 min Alcohol 70%

1 min Bleach 5%

Sterile dH_2O (3X, 2 min)

Adult grinding

Inoculation

Solid medium — **Liquid** medium

MMN with and without FB

LB with and without FB

Genetic characterization

- 16S rRNA sequencing
- BOX-PCR (primer BOXA1R)

LB - Luria Bertani
MMN - Modified Melin-Norkrans
FB - Fetal bovine

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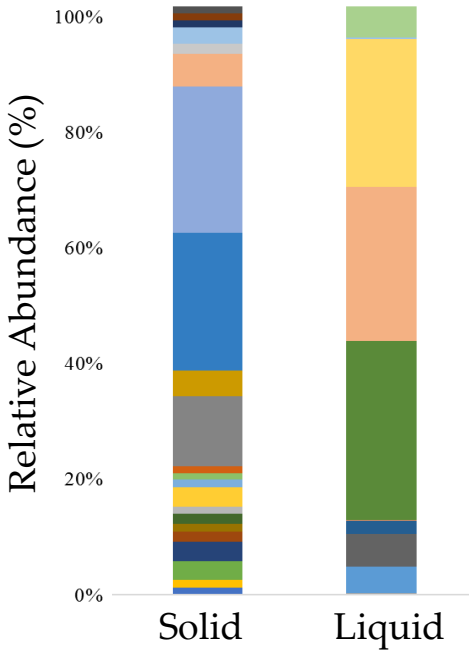
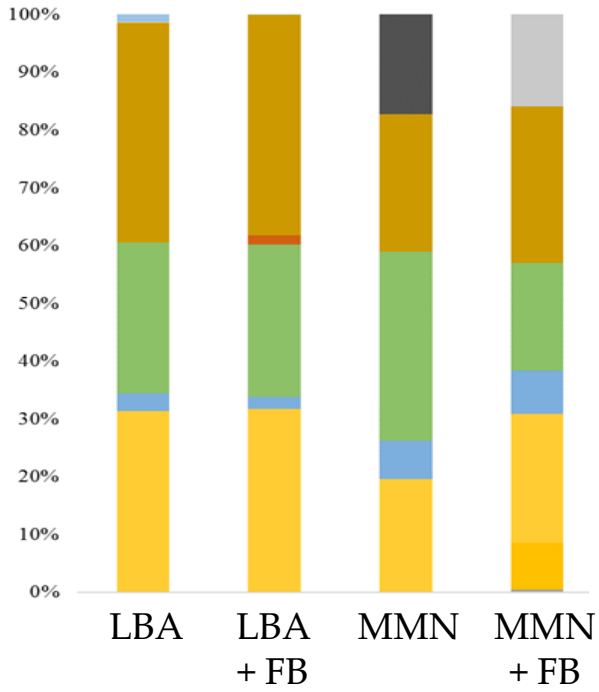
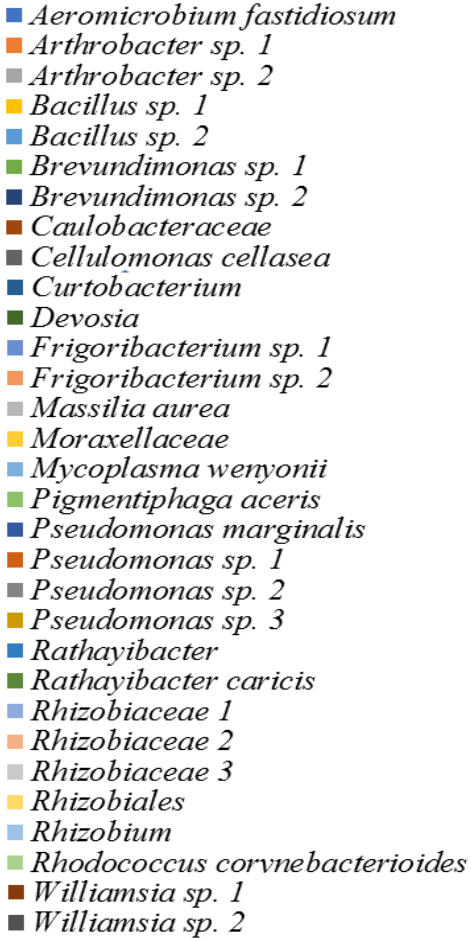
Results and Discussion

Solid vs. Liquid media



Type of culture media





- The solid media facilitated the growth of more diverse bacterial taxa

- No differences on the diversity of bacteria among the two media
- The addition of FB leads to a slight increase in bacterial abundance

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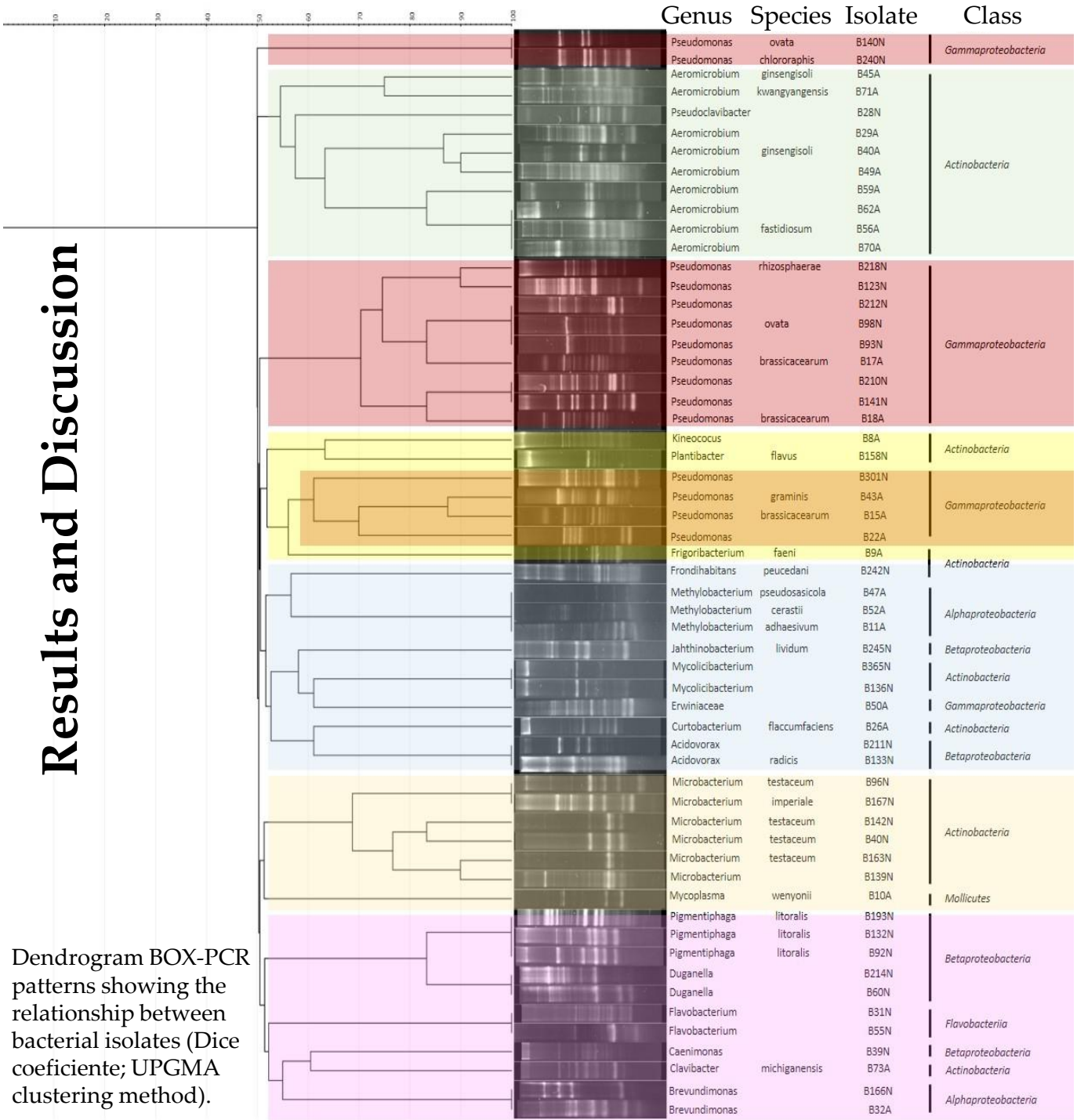
Results and Discussion



Dendrogram BOX-PCR patterns showing the relationship between bacterial isolates (Dice coeficiente; UPGMA clustering method).

The **BOX-PCR** revealed a high discriminatory power, allowing the differentiation of the bacteria at the intra-species level

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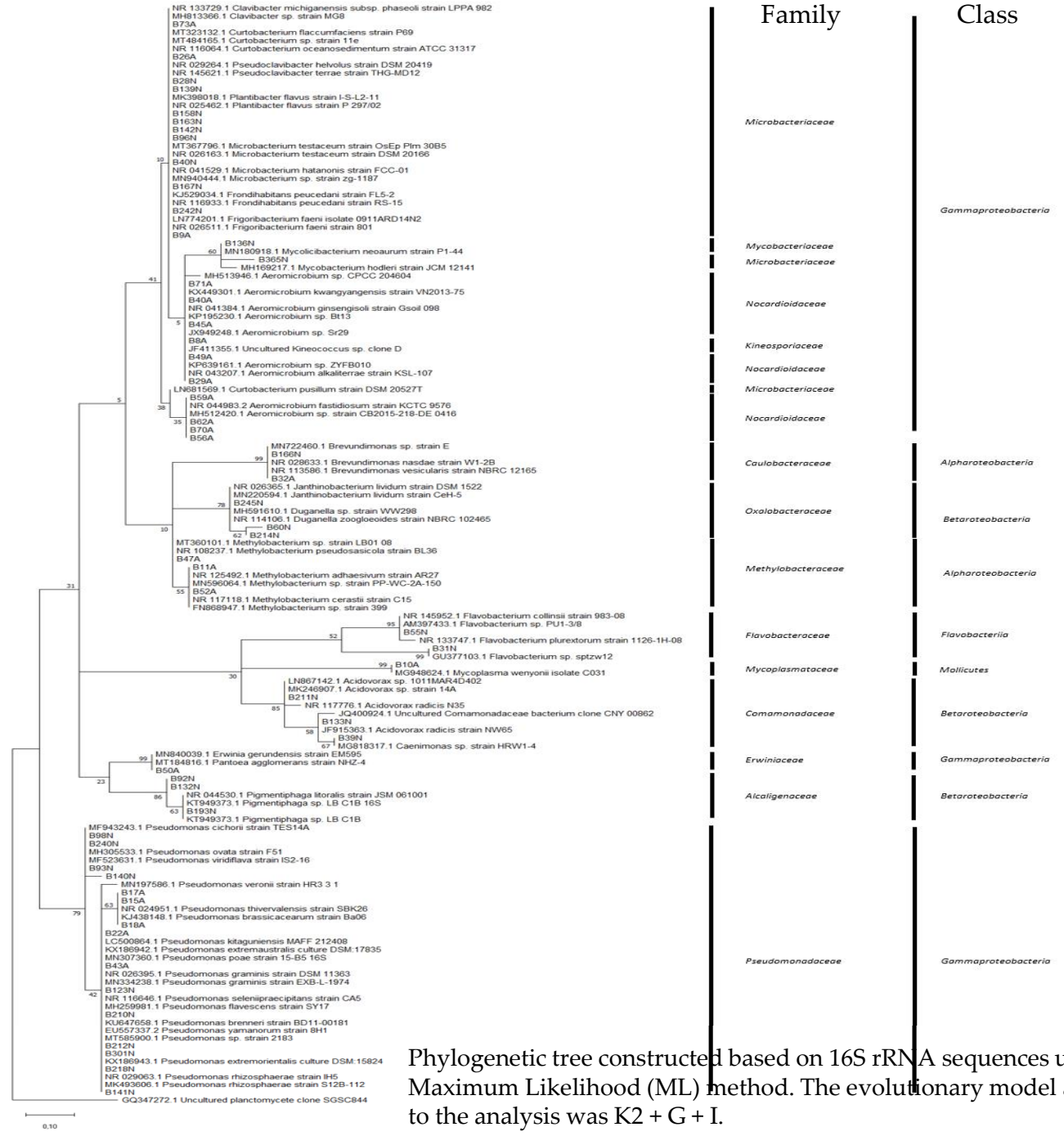
Results and Discussion



Phylogenetic tree constructed based on 16S rRNA sequences using the Maximum Likelihood (ML) method. The evolutionary model applied to the analysis was K2 + G + I.

16S rRNA gene sequencing method is more suitable in phylogenetic evaluations, generally grouping isolates belonging to the same genus

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Conclusions

- The exclusive growth of some species in MMN or LB, indicate that both media are complementary
- The BOX-PCR has a higher discriminatory power than 16S rRNA gene
- Clustering of the isolates using BOX-PCR fingerprinting was different to that obtained from the 16S rRNA gene phylogenetic tree
- Futures studies should evaluate the function of these microorganisms in *P. spumarius*.

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