Use of a novel MLVA assay as a promising genotype tool for the identification and tracking of P. savastanoi population
Pseudomonas savastanoi.

- Disease caused by its pathovars (A, B, C).
- Classification of pathovars (host range and genetic information).
- Discrimination among the strains based on phenotypic and genotypic methods.
- MLVA is very promising typing tool, with great discriminatory power, reliable and able to reveal the relationships at a micro-evolutionary level.

The aim of this study is to set up a novel MLVA assay to discriminate strains of P. savastanoi at inter- and intra-pathovar level and validate its accuracy and reliability.
Material and methods

- Genome analysis and using Tandem Repeats Finder program (Benson, 1999).
- Designing of primers and DNA amplification.
- Capillary Electrophoresis system to Determine the amplified fragments.
- To assess genetic relationships between haplotypes and associated strains, Minimum Spanning Tree (MST) and Neighbour Joining (NJ) dendrogram were obtained by Phyloviz 2.0 software.

Table 1. Assignment of isolates to HTs and details about their geographic and time origin.
Results and conclusion

Results
- Genome analysis has revealed 110 TR, but 20 were chosen.
- Eliminate of 5 TR due to amplification failure, production of multiple amplicons, or lack of reproducibility.
- A total of 46 distinct haplotypes with 2-9 TR loci differences
- The MLVA assay was able to trace the 3 pathovars under investigation and to discriminate different strains within the same pathovar (geographic origin and date of isolation)

Conclusion
- High discriminatory power, useful for identification and tracing specific types of the bacterium.
- To improve the method:
  - New VNTR identification and use of multiplex PCR.

Figure 1. Minimum Spanning Tree (MST) calculated using goeBURST + Euclidean distance algorithm. The circles represent the 46 haplotypes (HTs) as in Table 1; the pathovars are differentiated by colors (savastanoi = blue; fraxini = red; and nerii = green); the exact number of differences between haplotypes is reported on connecting lines.

Figure 2. NJ tree according to Saitou-Nei criterion. The haplotypes are represented in different colors according to their geographic origin (for details, check n° in Table 1). The two circles enclose nerii (lower left) and fraxini (upper right) strains.
Comparison of *Pseudomonas savastanoi* pathovars using 10 core genes by MLST Method.

Study the presence of 42 type three effectors in the three pathovars (*Savastanoi, Nerí, Fraxini*)…………………..

Work in progress:
Thank You

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