Optimisation of methods for the identification of '*Candidatus* Phytoplasma asteris' in *Croton bogotanus* plants using *leuS* and *secA* genes

Since 2006 a decline in the health status of many species of trees has been observed in the city of Bogota, Colombia, with significant losses of many urban trees in parks and alongside highways, mainly due to the presence of symptoms associated with phytoplasma diseases. The Plant Biotechnology Laboratory of the Military University Nueva Granada (Cajicá-Colombia), under the supervision of Dr Liliana Franco-Lara, has been studying phytoplasmas in these trees and the insect vectors, and the effect of the dispersion of these pathogens into crops around the city.

To achieve these goals, improvements to the standard diagnostic protocols are required to provide reliable methodologies to detect phytoplasmas. The PCR amplification of the 16S rRNA gene has been previously standardised, but is often insufficient for providing good discrimination between subgroups ('strains') of phytoplasmas, making it difficult to determine the precise host range and vectors for the different subgroups. One of the objectives of my MSc thesis is to standardise PCR tests based on other genes such as *amp, tuf, secY*, *secA* and others to improve the capacity for these to provide discrimination between different subgroups.

Last July I had the opportunity, funded by BSPP, to receive training in phytoplasma diagnostic techniques for a month in the laboratory of Professor Matt Dickinson at the University of Nottingham, UK. The purpose of the visit was to analyse DNA samples from *Croton bogotanus* trees from Bogota infected with phytoplasmas. The idea was to test them using PCR amplification of genes different to 16SrRNA such as the leucyl tRNA synthetase (*leuS)* gene and *secA* (for which universal primers had been developed at Nottingham). The samples were also analysed using the real-time Loop-Mediated Isothermal Amplification (LAMP) technology as part of the process of developing this technology for rapid phytoplasma detection.

Our results were unexpected and this actually opens new questions about the diagnostic methods and the sequence conservation of the Colombian phytoplasmas in comparison to other more studied phytoplasmas. The samples that we analysed using LAMP had been previously tested using the standard 16S rRNA primers, followed by RFLP and sequence analysis. Using LAMP, we confirmed the presence of phytoplasmas of group 16SrI and we found sequences that may belong to 16SrVII. We were also able to amplify the *leuS* gene from infected samples carrying 16SrI and 16SrVII subgroups; however, there were also a number of samples where we were unable to detect any phytoplasma DNA, despite the samples coming from trees showing clear phytoplasma disease symptoms. The reasons for this are unclear, and may reflect difficulties in getting good quality DNA from woody plant hosts that doesn't contain enzyme inhibitors. It may also reflect the known uneven distribution of phytoplasmas in plant phloem, and the degradation of DNA in samples during transit and storage between Colombia and the UK. These questions need to be addressed in development of reliable phytoplasma diagnostics from woody plants.

Despite my language difficulties my experience visiting the UK was excellent. Nottingham is a welcoming place with some spectacular landscapes and beautiful architecture, and I also had the chance to visit London, which is a beautiful city. I had the opportunity to share ideas, knowledge, techniques and research results with other phytoplasma researchers which enriched my work and my MSc thesis. The people at Nottingham were friendly and helpful during my stay. Overall, I had a very good personal experience because this was the first time I had left my country, and I am immensely thankful to the community at the Sutton Bonington Campus.

I thank the BSPP for the financial support for my visit at the University of Nottingham. It enhanced my knowledge of new methodologies such as LAMP, which is a quick and reliable technique for the detection of phytoplasmas in plant samples. All this knowledge will be transferred to our laboratory in Colombia and these results will be included as part of my thesis.

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