QBOL - Identification of phytoplasmas using DNA 'barcodes'

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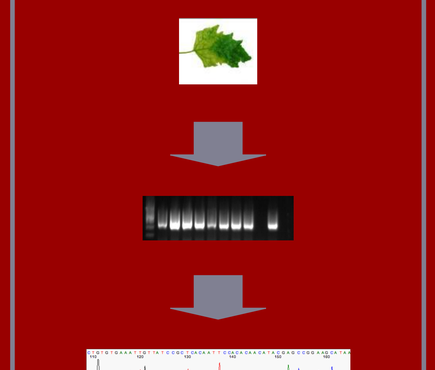
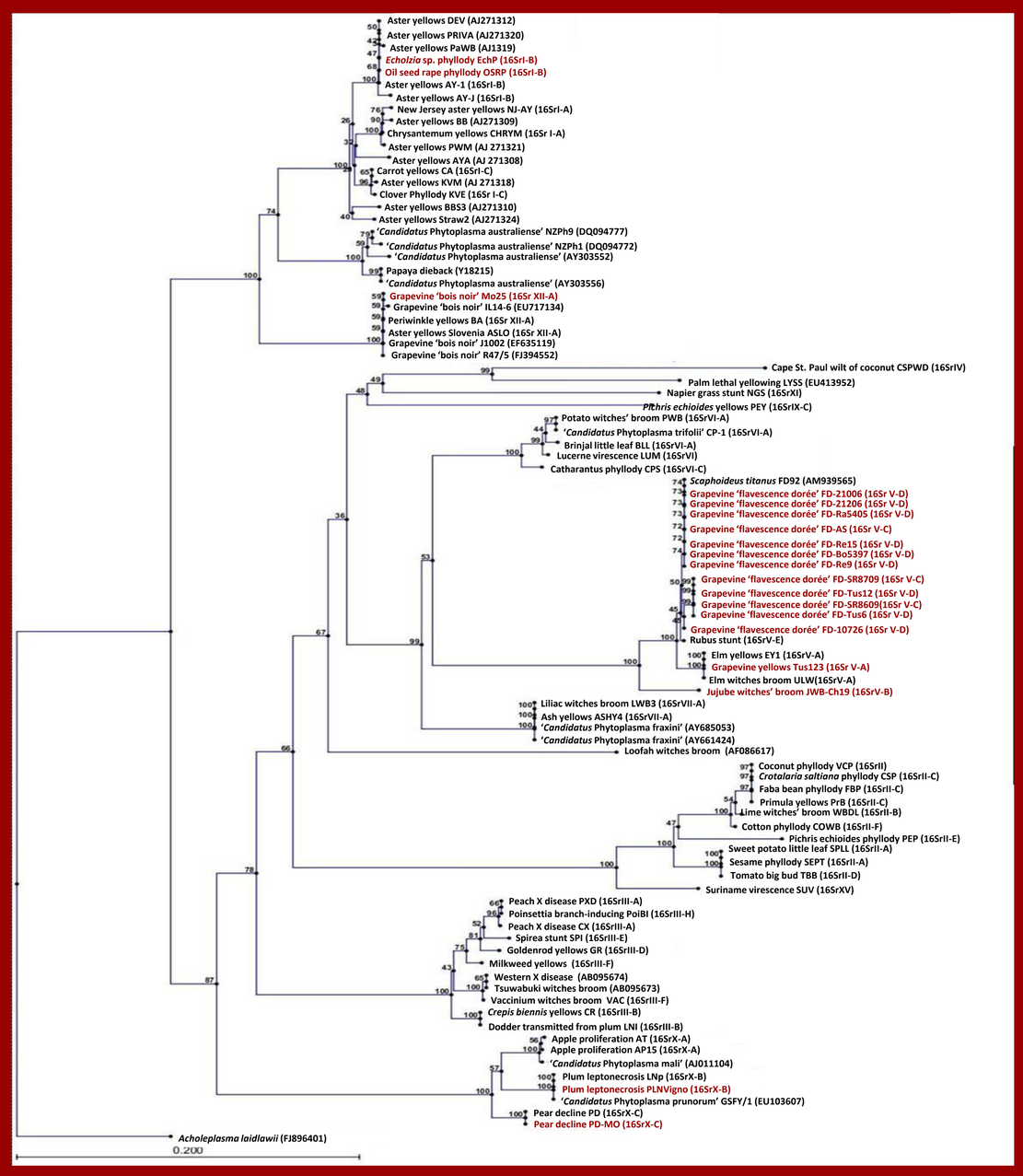


Climate change, expansion of the EU and the increase of international trade may facilitate the spread of phytoplasma associated diseases, therefore a quick and handy system for correct plant pathogen identification is in great demand. DNA barcoding has arisen as a robust and standardised approach to species identification. QBOL, a new project funded by EU FP7 aims to create a public reference database of diagnostic DNA sequences for all quarantine plant pathogens and to make it available for plant health diagnosticians. Some of the regions used in QBOL are formally approved BARCODE regions (e.g., COI in insects). Approval of other standard regions is in progress.

Phytoplasma 'barcoding' has been performed for many years, particularly using the 16S rDNA, but also other genes such as secY, secA, tuf and ribosomal proteins; however most of these regions span more than 1 kb and/or primers are not generic, which make them impractical for routine 'barcoding' of unknown phytoplasmas. Available sequences of elongation factor Tu (Tuf) and 16S genes were explored for selecting regions suitable for phytoplasma DNA 'barcoding' to develop robust markers of a size that can easily be sequenced (400-600 bp) and that can be obtained from most, if not all phytoplasma ribosomal groups and/or '*Candidatus* Phytoplasma' species using generic primers. A number of phytoplasma strains maintained in periwinkle and field collected were used for PCR amplification with newly developed primers for Tuf and 16S regions and then sequenced. The 5' end of the Tuf and the 5' end of 16S genes were used for 'barcoding'. Sequences of approximately 450 bp for Tuf and 625 bp for the 16S gene were obtained from more than 60 phytoplasma strains, belonging to 12 different 16Sr groups. Using these sequences as 'barcodes' it was possible to identify the phytoplasmas into '*Candidatus* species' or 16Sr groups. The obtained sequences will be available in the newly developed QBOL database.

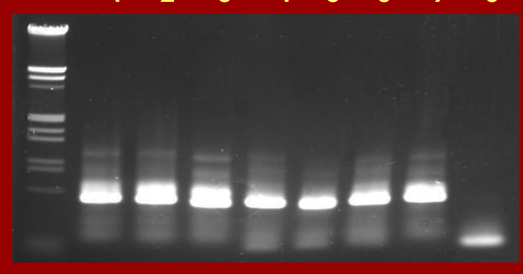


Phylogenetic tree of Tuf 400/835 sequences from this study and from GenBank (accession numbers in parantheses). *Acholeplasma laidlawi* tuf sequence is used to root the tree. In red phytoplasma detected in field collected samples, strains sequenced in this work without GenBank number.

16S rDNA fragment

Preliminary studies suggested that the 5' end of 16S shows the most variability within phytoplasmas.

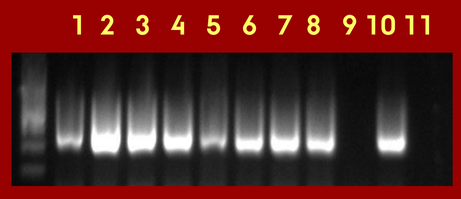
A fragment in the 5' end of 16S was amplified using P1 and a reverse primer designed in this study, P625r, resulting in a product of app. 625 bp. The primers were able to amplify phytoplasma DNA from all tested groups (16Sr-I, -II, -III, -IV, -V, -VI, -VII, -IX, -X, -XI, -XII and -XV) and did not amplify plant DNA.



Nested PCR amplification of different phytoplasma strains using Tuf340/Tuf890 followed by Tuf400/Tuf835 primers. 1, PEY 16SrIX-C; 2, CP-1 16SrVI-A; 3, ULW 16SrV-A; 4, PD 16SrX-C; 5, SUV 16SrXV; 6, CSPWD 16SrIV; 7, NGS 16SrXI, and 8 negative control

Translation elongation factor Tu (Tuf)

Tuf has mainly been used for strain differentiation inside some ribosomal groups such as the 16SrXII (Stolbur) group. On the basis of published sequences degenerated nested primers Tuf340/Tuf890 followed by Tuf400/Tuf835 were designed. These primers were able to amplify strains from groups 16Sr-I, -II, -III, -IV, -V, -VI, -VII, -IX, -X, -XI, -XII and -XV. This Tuf fragment of 367-391 bp provides good resolution among phytoplasma groups already designed on 16Sr gene as can be seen in the phylogenetic tree.



PCR amplification of different phytoplasma strains using P1/P625r primers. 1, CHRYM 16SrI-A; 2, SPLL 16SrII-D; 3, PXD 16SrIII-A; 4, EY 16SrV-A; 5, CP-1 16SrVI-A; 6, AP15 16SrX-A; 7, ASLO 16SrXII-A; 8, PEY 16SrIX-C; 9, BVK 16SrXI-C; 10, ASHY 16SrVII-A, and 11, negative control

Discussion

These preliminary results show that it is possible to develop small DNA ' barcodes' for use in phytoplasma identification. PCR amplification from 16S and Tuf was obtained from a range of strains belonging to groups 16Sr-I, -II, -III, -IV, -V, -VI, -VII, -IX, -X, -XI, -XII and -XV. Using a combination of the sequences it was possible to differentiate the investigated 16Sr groups and also to differentiate subgroups in wich epidemiologically or quarantine relevant phytoplasmas are classified such as. 16Sr groups -V and -XII.

Future work

Production of 16S and Tuf ' barcode' sequences from a large number of strains from all available groups will be produced. 'Barcode' sequences will be deposited in a QBOL Database for public access.

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SEVENTH FRAMEWORK PROGRAMME