Genes encoding collagen-like repeats are promising Variable Numbers Tandem Repeats (VNTR) markers for the differenciation of Bois noir-associated "Candidatus Phytoplasma solani" strains

Pascal Salar, Christina Mortada, Fouad Jreijiri, Elia Choueiri, Martina Šeruga Musić, Xavier Foissac

Genes encoding collagen-like repeats are promising Variable Numbers Tandem Repeats (VNTR) markers for the differenciation of Bois noir-associated '*Candidatus* Phytoplasma solani' strains

Pascal Salar[1], Christina Mortada[2], Fouad Jreijiri[2], Elia Choueiri[2], Martina Seruga Music[3], **Xavier Foissac**[1]

[1]*University of Bordeaux, INRAE, Fruit Biology and Pathology, Villenave d'Ornon, France*
[2]*Lebanese Agricultural Research Institute, Department of Plant Protection, Tal Amara, Zahlé, Lebanon*
[3] *University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia*
**Corresponding author(s):* xavier.foissac@inrae.fr

INTRODUCTION

In population genetics, repeated DNA sequences are widely targetted to describe genetic diversity. In bacteriology, Variable Numbers of Tandem Repeats (VNTR) are widely used as such repeats do not vary at the same pace as do neutral genes (Hood et al., 1996; Frotingham and Meeker-O'Connell, 1998) and usually evolve in a *rec*A-independent manner (Puopolo *et al.,* 2001). When used in combination (Multiple Loci VNTR Analysis, MLVA) (Johanss0n et al., 2004), they produce fingerprints of bacterial populations, especially at the site of emergence which translate as a bottle-neck in terms of genetic variability. In the frame of a '*Candidatus* Phytoplasma solani' (CaPsol) genome survey of the PO strain, a gene, named *coll-like*, was found to encode GXY amino acid repeats reminiscent of collagen structure (Cimerman et al., 2006). Search for other GXY repeats in CaPsol strain PO draft genome revealed the presence GXY repeats in a gene encoding a surface protein. The encoded protein is also possessing large repeated domains upstream of the collagen-like repeats, a signal peptide and a C-terminal hydrophobic alpha-helix, a structure reminiscent of Vmp1 (Cimerman et al., 2009). This gene will be referred as *vmp3-coll.* The objective of this study was to assess the variability of these two potential VNTRs in grapevine Bois noir-associated CaPsol isolates.

MATERIALS AND METHODS

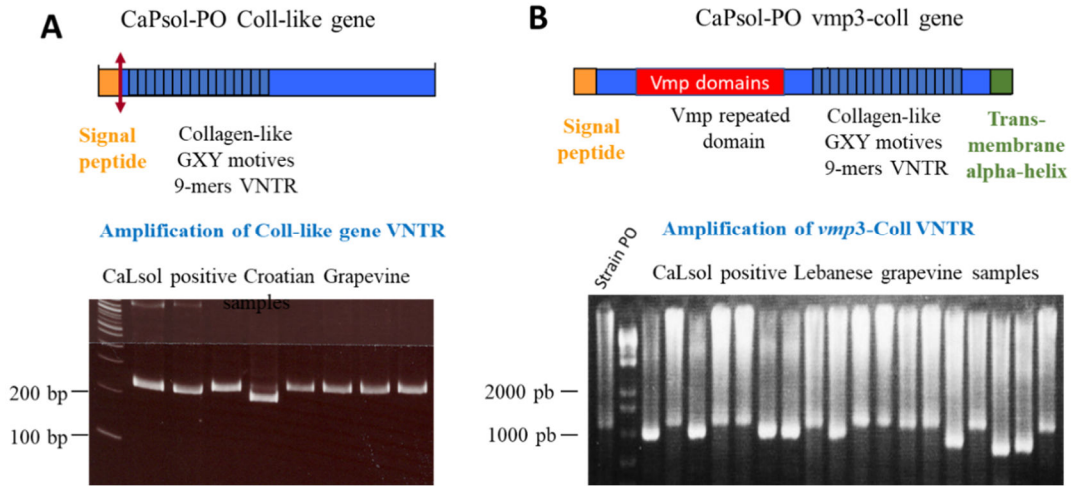
Total nucleic acids were extracted from 1 g of grapevine petioles according to CTAB standard procedure from samples collected in Bekaa valley (Lebanon) and from various location in Croatia. These samples had been shown to be positive for CaPsol infection (Mortada et al., 2013; Plavec et al., 2015). They were submitted to nested-PCR amplification with the primers described below. For *coll-like*, the first primer pair was collF1 (5'-CCTTTTATCATAACCTGTTT-3') / collR1 (5'-CAATAGGATATAGATAG-3'), followed by the primer pair collF2 (5'-GCTATTATTTATGCTGGCTC-3') / collR2 (5'-CGCTGTCCGTCTTTTGCTAA-3'), and PCR conditions were 95°C 2 min, 35 cycles of 95°C 30 sec, 55°C 30 sec, 72 °C 15 sec. For *vmp3-coll*, the first primer pair was vmp3-F5 (5'-GCTTCAAATAAGAATAGCATCAG-3') / vmp3-R4 (5'-GTTGCTGTATCTGGTGAAGT-3'), followed by vmp3-F4 (5'-CAACTAATT-TTGGACCTAACGG-3') / vmp3-R3 (5'-GTTTGTAGCTGGTTGATCTGG-3'), and PCR conditions were 95°C 2 min, 35 cycles of 95°C 30 sec, 55°C 30 sec, 72 °C 1 min. PCR products were analysed on 1.5 % and 0.7 % agarose gel electrophoresis for *coll-like* and *vmp3-Coll*, respectively.

RESULTS AND DISCUSSION

All selected CaPsol-associated Bois noir isolates from Croatia showed nested-PCR amplifications with *coll-like* primer pairs, with three different electrophoretic mobility profiles observed on gel electrophoresis among the eight Croatian samples tested (Figure 1-A). All selected CaPsol-associated Bois noir isolates from Lebanon showed nested-PCR amplifications with *vmp3-coll* primer pairs, with at least five different electrophoretic mobility profiles observed on gel electrophoresis among the

eighteen Lebanese samples tested (Figure1-B). For both genes, the sequencing of the obtained amplicons will indicate the actual number of VNTR repeats.

Figure 1: Structure of Coll-like and Vmp3-coll proteins and nested-PCR amplification of corresponding VNTR in Croatian (A below) and Lebanese grapevine samples (B below).



The amplification and sequencing of *vmp3-coll* VNTR among a set of sixteen CaPsol isolates from eleven Euro-mediterranean countries of the SEE-ERANET/ STOLBUR-EUROMED network (Foissac et al., 2013), indicated that the number of *vmp3-coll* VNTR was highly variable among CaPsol isolates and ranging from 28 to 64. Only three CaPsol isolates gave no *vmp3-coll* VNTR amplifications indicating either the absence of *coll-like* and *vmp3-coll* genes in some of the CaPsol strains tested or sequence variability at the site of primers.

ACKNOWLEDGEMENTS

This study was supported by the Plan National Dépérissement du Vignoble grant RENOV and the French-Lebanese bilateral project CEDRE 13E-L2.

REFERENCES

Cimerman, A., Arnaud, G., & Foissac, X. (2006). Stolbur phytoplasma genome survey achieved using a suppression subtractive hybridization approach with high specificity. *Applied and Environmental Microbiology*, 72(5), 3274-3283.

Cimerman, A., Pacifico, D., Salar, P., Marzachì, C., & Foissac, X. (2009). Striking diversity of *vmp1*, a variable gene encoding a putative membrane protein of the Stolbur Phytoplasma. *Applied and Environmental Microbiology*, 75(9), 2951-2957

Foissac, X., Carle, P., Fabre, A., Salar, P., Danet, J. L., & STOLBUR-EUROMED-consortium. (2013). '*Candidatus* Phytoplasma solani' genome project and genetic diversity in the Euro-Mediterranean basin. Invited conference. Paper presented at the Third European Bois Noir Workshop.

Frothingham, R., & Meeker-O'Connell, W. A. (1998). Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. *Microbiology-Uk*, 144, 1189-1196.

Hood, D. W., Deadman, M. E., Jennings, M. P., Bisercic, M., Fleischmann, R. C., Venter, J. C., et al. (1996). DNA repeats identify novel virulence genes in *Haemophilus influenzae*. *Proceedings of the National Academy of Sciences of the United States of America*, 93(20), 11121-11125

Johansson, A., Farlow, J., Larsson, P., Dukerich, M., Chambers, E., Byström, M., et al. (2004). Worldwide genetic relationships among *Francisella tularens*is isolates determined by multiple-locus variable-number tandem repeat analysis. *Journal of Bacteriology*, 186(17), 5808-5818.

Mortada, C., Jreijiri, F., Choueiri, E., & Foissac, X. (2013). Genetic diversity of bois noir phytoplasma in two vineyards of the Bekaa valley Lebanon. Paper presented at the Third European bois noir workshop, Barcelona.

Plavec, J., Krizanac, I., Budinscak, Z., Skoric, D., & Music, M. S. (2015). A case study of FD and BN phytoplasma variability in Croatia: multigene sequence analysis approach. *European Journal of Plant Pathology*, 142(3), 591-601.

Puopolo, K. M., Hollingshead, S. K., Carey, V. J., & Madoff, L. C. (2001). Tandem repeat deletion in the alpha C protein of group B *Streptococcus* is *rec*A independent. *Infection and Immunity*, 69(8), 5037-5045.