Development of a DNA-based real-time PCR assay to quantify Allorhizobium vitis overtime in grapevine plantlets

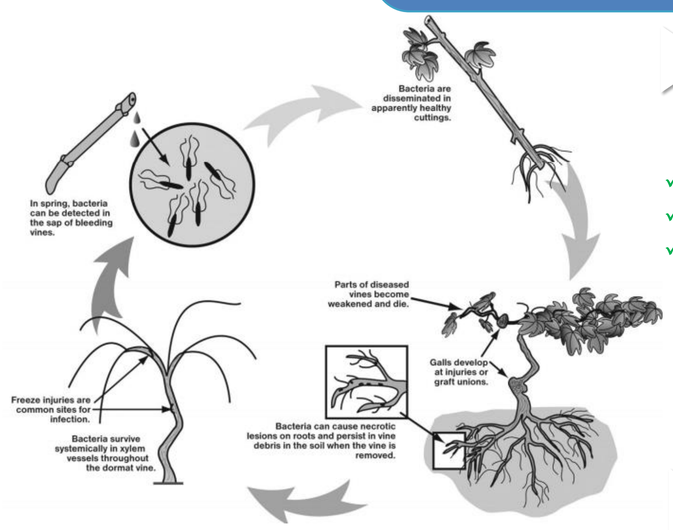
Nguyen Ht, Doré J, Essaid Ait Barka, Lavire C, Clement C, Vial L, Sanchez L

DEVELOPMENT OF A DNA-BASED REAL-TIME PCR ASSAY TO QUANTIFY *ALLORHIZOBIUM VITIS* IN GRAPEVINE PLANTLETS

Nguyen-Huu Trong[1], Doré Jeanne[2], Ait Barka Essaid[1], Lavire Céline[2], Clément Christophe[1], Vial Ludovic[2], Sanchez Lisa[1]

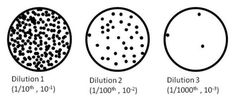
[1] Unité EA 4707 Résistance Induite et Bioprotection des Plantes (RIBP), URCA, Reims, France
[2] UMR Ecologie Microbienne, CNRS, INRA, VetAgro Sup, UCBL, Université de Lyon, Lyon, France
Contact: huu-trong.nguyen@univ-reims.fr

Background



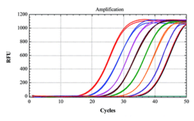
- **Crown gall**: important disease of grapevine worldwide, causing yield decrease and even plant death (Burr et al. 1998; Schroth et al. 1988)
- Causal agent: ***Allorhizobium vitis*** (previously named *Agrobacterium vitis,* Mousavi et al. 2014)
- Risk of spreading and persisting in vineyard (Kuzmanović et al. 2018) → **need of detection** of pathogen
- Study the interaction host-pathogen → **need of quantification** of bacterial population

PLATE COUNTING



- ✓ Simple
- ✓ Reliable
- ✓ Quantify living bacterial cells
- × Non-storable samples
- × Contamination
- × Technical repetitive mistakes
- × Risk of false-negative
- × Time-consuming
- × Intensive labor

DNA-BASED qPCR



- ✓ Storable samples
- ✓ Sensitive
- ✓ Accurate, specific
- ✓ Rapid
- × No distinction between living/dead cells
- × Risk of overestimation

(Ross and Somssich 2016)

Methods

Objectives

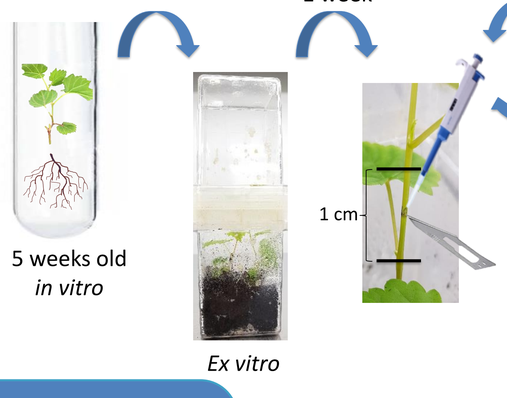
Develop DNA-based qPCR as an alternative method for ① detection and ② quantification of *A. vitis* in grapevine plantlets by comparing and validating with plate counting assays

1 Primer pairs

Avi_1889	5'-TTCTGCATCGACCCGGAAAT-3' / 5'-GCGAAACATCCGCTCCAAAA-3'	5'-ATTGGAATATCTGTCCCG-3' / 5'-GGCGAGATCGCGGATATT-3'	virD59
Avi_5072	5'-CGTCTGGCTGATCTTGGGTT-3' / 5'-GATTGATCGCTTCCTCCGCT-3'	5'-GGTGATGGTCCAGCATCCAA-3' / 5'-AAGCCAAACATTTTCCGCCC-3'	virD3
Avi_7106	5'-CGGCGGAACACCCTTCATTA-3' / 5'-ACCGTTGGCATGATCTCGAA-3'	5'-AATCCGGAGGTGATGGT-3' / 5'-GGCGTCATGTAAGCGTTG-3'	virD3cons

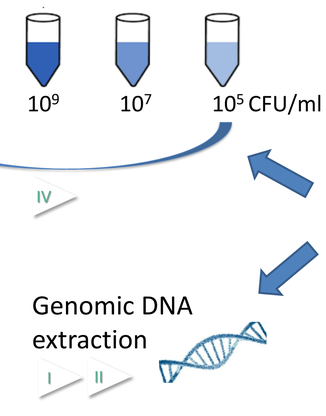
2 Plant material

Grapevine *Vitis vinifera* cv. Chardonnay



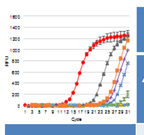
3 Microorganisms

***A. vitis* strain S4**



Results

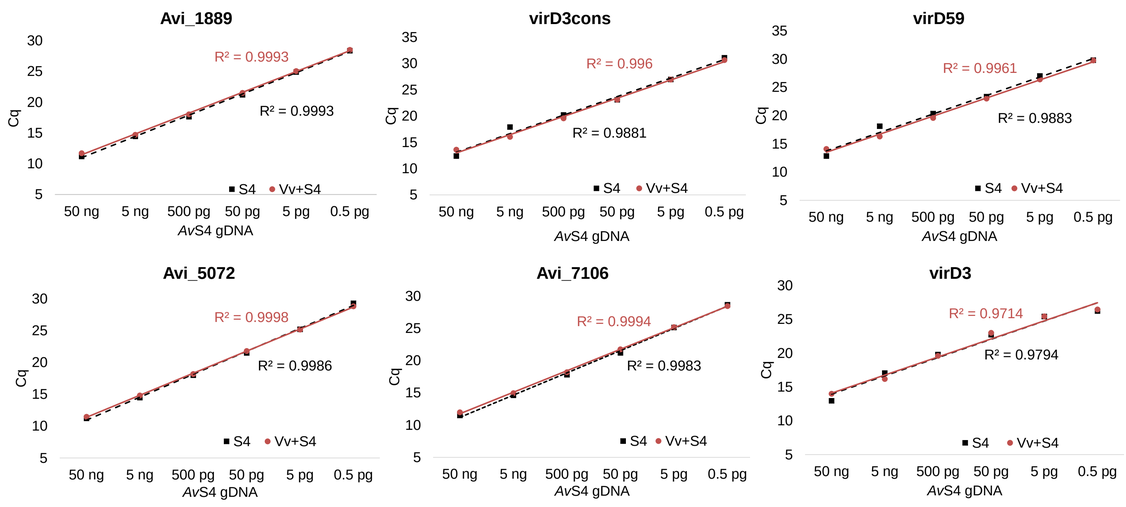
I Primer specificity



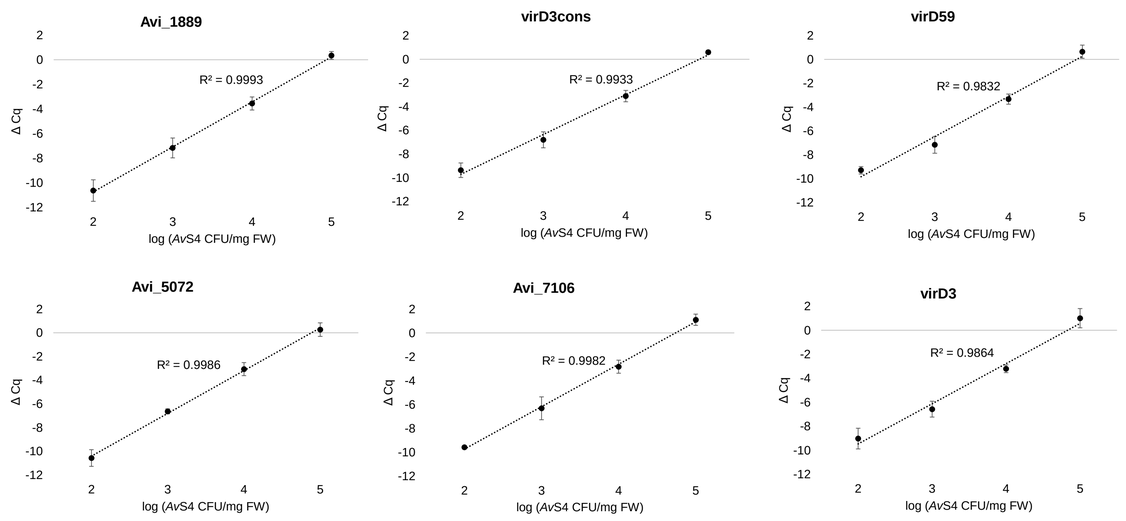
	A. vitis strains					Non-*A. vitis spp.*					
	A. vitis S4	*A. vitis* BT2-2	*A. vitis* BT3-1	*A. vitis* ET2-10	*A. vitis* KT1-1	*A. fabrum* C58	*A. rhizogenes* K84	*B. thailandensis*	*E. coli*	*P. brassicacearum* NFM 421	*S. meliloti* 1021
Avi_1889	✓	✓	✓	✓	✓	×	×	×	×	×	×
Avi_5072	✓	✓	✓	✓	✓	×	×	×	×	×	×
Avi_7106	✓	×	×	✓	×	×	×	×	×	×	×
virD3	✓	×	×	×	×	×	×	×	×	×	×
virD59	✓	×	×	×	×	×	×	×	×	×	×
virD3cons	✓	✓	✓	✓	✓	×	×	×	×	×	×

➢ Primers are specific for *A. vitis* S4 and some other strains of *A. vitis*.

II Primer sensitivity

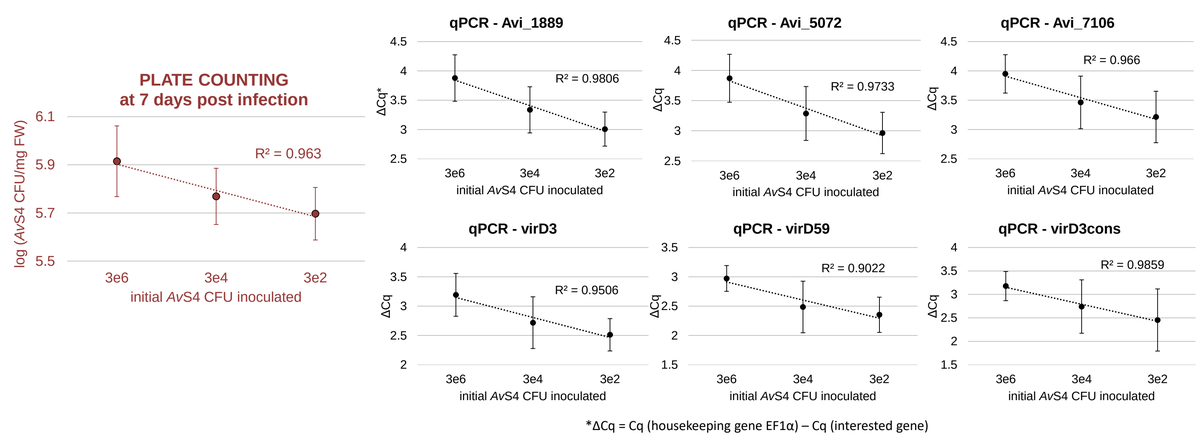


➢ Primers can quantify different levels of biomass of *A. vitis* S4.
➢ Their activity is not influenced by plant gDNA matrix.



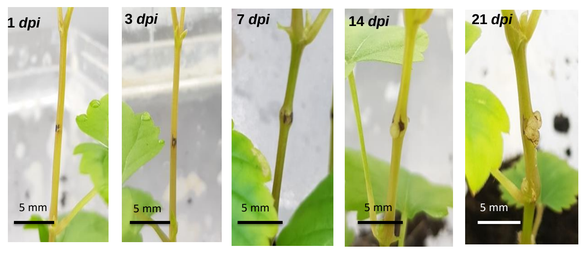
➢ Primers can detect until 300 *A. vitis* S4 cells per mg grapevine stem fresh weight.

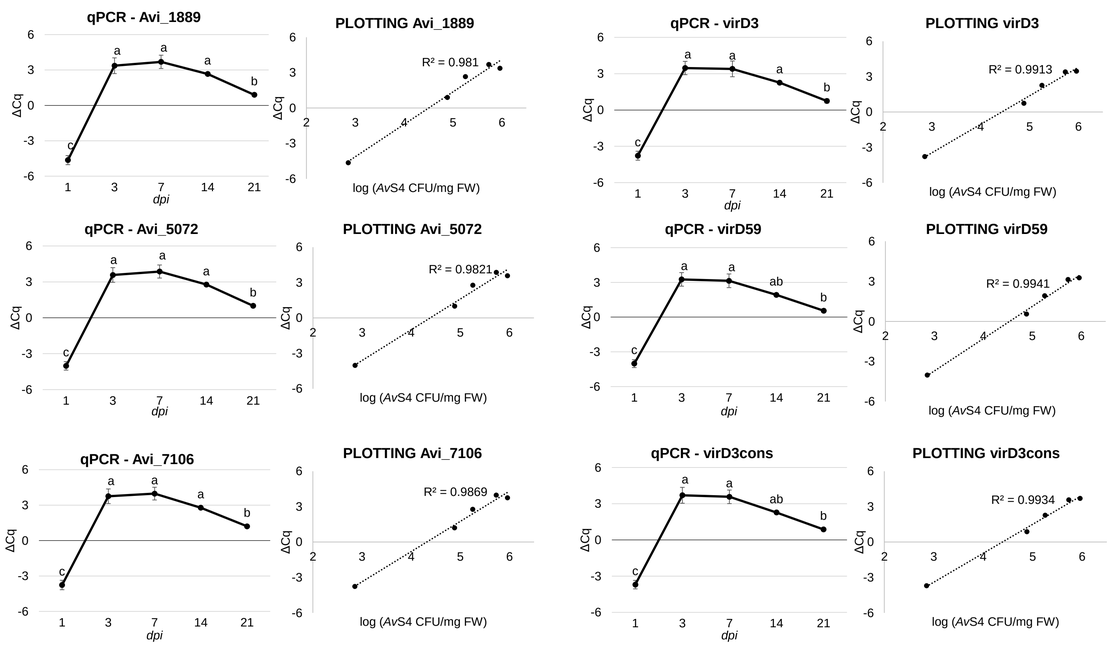
III Quantification of *A. vitis* S4 populations infected from different quantities



*ΔCq = Cq (housekeeping gene EF1α) – Cq (interested gene)

IV Monitoring *A. vitis* S4 populations in grapevine plantlets





*ΔCq = Cq (housekeeping gene EF1α) – Cq (interested gene). Same letters indicate no significant difference (Tukey's test, $p < 0.05$)

➢ qPCR method gave similar results as plate counting assays in quantifying *A. vitis* S4 populations in grapevine plantlets over time.

Conclusion

qPCR as the alternative method for quantifying *A. vitis* S4 in grapevine plantlets thanks to its accuracy, rapidity, flexibility, sensitivity and specificity over plate counting assay.

Acknowledgement

Grand Est ALSACE CHAMPAGNE-ARDENNE LORRAINE
l'Europe s'engage en Champagne-Ardenne
UNION EUROPÉENNE Fonds Européen de Développement Régional

References

- Burr, T. J., Bazzi, C., Süle, S., and Otten, L. 1998. Crown gall of grape: Biology of *Agrobacterium vitis* and the development of disease control strategies. Plant Dis. 82:1288–1297.
- Kuzmanović, N., Puławska, J., Hao, L., and Burr, T. J. 2018. The ecology of *Agrobacterium vitis* and management of crown gall disease in vineyards. Curr. Top. Microbiol. Immunol. :15–53.
- Mousavi, S. A., Österman, J., Wahlberg, N., Nesme, X., Lavire, C., Vial, L., et al. 2014. Phylogeny of the *Rhizobium-Allorhizobium-Agrobacterium* clade supports the delineation of *Neorhizobium* gen. nov. Syst. Appl. Microbiol. 37:208–215.
- Ross, A., and Somssich, I. E. 2016. A DNA-based real-time PCR assay for robust growth quantification of the bacterial pathogen *Pseudomonas syringae* on *Arabidopsis thaliana*. Plant Methods. 12.
- Schroth, M. N., McCain, A. H., Foott, J. H., and Huisman, O. C. 1988. Reduction in yield and vigor of grapevine caused by crown gall disease. Plant Dis. 72:241–246.