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A DNA-capture approach for detection and genome-wide sequencing of Flavescence dorée phytoplasma

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INTRODUCTION

Flavescence dorée phytoplasma (FDp) is a non-cultivable quarantine bacterial pest causing outbreaks in European vineyards where it is transmitted by the leafhopper *Scaphoideus titanus* (Tramontini et al., 2020). FDp can be detected by real-time PCR assays and FDp strains can be genetically characterized by Multilocus sequence typing (Arnaud et al., 2007). However, the genetic characterization of FDp is often limited due to the low amount of FDp DNA in grapevine nucleic acid. Recently, a DNA capture approach was described to enrich DNA of the citrus pathogen "*Candidatus* Liberibacter asiaticus" prior to Illumina sequencing (Cai et al. 2019). We describe the design of a SureSelect (Agilent) RNA probe system to capture FDp DNA. The FDp enrichment probe consisted of RNA probes covering all coding sequences (CDS) of the FD92 FDp genome (Carle et al., 2011). RNA probes were preferred to take advantage of the high strength of RNA/DNA hybridisation expected to compensate the lower capture efficiency due to the low GC % in the FDp genome (21.7%). This approach was applied to DNA extracts of field -collected or insect-inoculated FDp-infected grapevines. We present here the outcome in terms of enrichment and sequencing coverage for pure extracts and for serial dilutions mimicking decreasing rates of infection.

MATERIALS AND METHODS

The samples VISa and VISb were Cabernet-Sauvignon grapevine plants inoculated with FDp genotype M54 using infectious *S. titanus* under greenhouse-controlled conditions (Eveillard et al., 2016). The sample VIT was Cabernet-Sauvignon infected with M54 collected in vineyards in Faleyras (Gironde, France). Total nucleic acids were extracted from 1.5g of petioles according to standard procedure (Maixner et al., 1995), treated with 1 µg/µl RNAse A for 30 min at 37°C and purified on Promega Wizard® SV Gel and PCR Clean-Up System columns. DNA concentration was measured with Qubit-4 fluorimeter. The number of FDp genome copies per µl of DNA extract was measured by qPCR (Eveillard et al. 2016). Serial dilutions in healthy grapevine DNA were prepared. Probes consisted of (i) 560 CDS and (ii) 16S and 23S rRNAs of the genome of strain FD92 of genotype M54 and vmpA and B cluster II which predominates in European vineyards, (iii) *vmp*D and *vmp*E adhesin gene sequences specific to strain FD-CAM05 of genotype M50 and (iv) *vmp*A, *vmpB* and *imp* genes representative of the FDp strains diversity. A total of 48649 RNA probes long of 120 nucleotides covering 495524 bp with tiling coverage of 3X were synthetized by Agilent. Samples were processed according to "SureSelectXT HS target enrichment system for Illumina multiplexed sequencing platforms" Agilent manual version D0 (August 2020). Paired-end Illumina sequencing 2 x 150 bp was performed on a NextSeq 2000 at the Plateforme Génome Transcriptome de Bordeaux (PGTB). Sequences were trimmed to eliminate adaptor sequences. Sequences with length shorter than 50 bp or quality below phred20 were eliminated. Finally, sequences were mapped to the FD92 FDp genome using Bowtie 2 and mapping results analyzed with Samtool coverage under Galaxy.

RESULTS AND DISCUSSION

The selected samples were highly infected. According to qPCR, VIS and VIT grapevine DNA extract contained 0.754 % and 0,46 % of FDp DNA (Table 1). After SureSelect enrichment, 90.34 % and 84.69 % of the sequence reads corresponded to FDp for VIS and VIT pure extracts. The enrichment was of 120 for VIS and of 184 for VIT. The

enrichment was even higher with all the 1/16 serial dilutions of both VIS and VIT, indicating that the capture system was saturated in the case of pure extracts. The coverage of mapping to the FDp genome was higher than 72 % for pure extracts, the 1/16 dilutions of VIS and VIT and for the 1/256 dilution of VIS. It must be noted that the probes are covering 75 % of the 647 kbp of the FD92 FDp genome. Therefore, higher coverage indicated that intergenic sequences bordering the RNA probes were partly captured. For higher dilutions of VIS and VIT, the coverage was limited to 24.3 % and 45.3 % respectively. Depth of 329 and 224 however indicate that some parts of the genome were more efficiently captured. These results indicate the efficiency of the SureSelect RNA probe capture system to enrich grapevine DNA in FDp DNA and give sufficient data for a genome-wide genetic characterization of FDp strains. In order to reduce costs, the enrichment was also evaluated with capture probes diluted at 1/8 in comparison with pure probes, on serial dilutions of infected grapevine extracts (VITb) prepared as described above. For FDp DNA loads higher than 0,04 %, enrichment, coverage and depth showed high values for both conditions (Table 2).

Table 1: Statistics of Illumina sequencing after enrichment capture on FDp SureSelect probes.

Samples	Total reads (million)	Reads mapped (million)	Coverage (%, ≥30X)	Depth (X, ≥30X)	% of FDp reads after capture (A)	% of FDp DNA in initial extract (B)	Enrichment A/B
VISa pure	46,84	42,31	84.5	11162	90,34	0.758 (1)	119
VISa dil 16	25,04	11,05	82.2	3059	44,14	0.034 (2)	1284
VISa dil 256	22,61	1,40	72.2	436	6,21	0.0021 (2)	2891
VIS dil 4096	24,75	0,40	24,3	329	1,64	0.00013 (2)	12214
VIT pure	55,34	46,86	85.4	12468	84,69	0,466 (1)	182
VIT dil 16	21,44	3,88	78.0	1229	18,10	0.0073 (2)	2487
VIT dil 256	19,49	0,48	45.3	224	2,48	0.00046 (2)	5451

(1) Evaluated by qPCR, (2) Calculated from the dilution factor in healthy grapevine DNA

Table 2: Statistics of sequencing after enrichment capture on FDp SureSelect probes pure vs 1/8 diluted.

Samples	Total reads (million) pure/diluted	Coverage (%, ≥30X) pure/diluted	Depth (X, ≥30X) pure/diluted	% of FDp reads after capture (A) pure/diluted	% of FDp DNA in initial extract (B)	Enrichment A/B pure/diluted
VISb pure	78,07/33,4	82.9/81,4	18722/8545	89/92,8	0.51 (1)	174/182
VISb dil 16	15,3/22,5	83,2/84,6	4123/5981	42,1/67,62	0.041 (1)	1027/1649
VISb dil 256	31,5/19,5	72,5/45,3	656/224	6,9/2,5	0.0045 (1)	1537/551

(1) Evaluated by qPCR

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