

# **Genome sequence of *Plasmopara viticola* and insight into the pathogenic mechanism**

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## Supporting information

**Supplementary Table S1.** Summary of sequencing data information of *P. viticola* isolate ‘JL-7-2’.

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**Supplementary Table S4.** BUSCO analysis of the genome assembly.

**Supplementary Table S5. Transcription of predicted *P. viticola* genes during infection.** Number of RNA-Seq reads detected in RNA extracted expressed genes of *P. viticola* isolates ‘JL-7-2’, ‘ZJ-1-1’ and ‘CSIRO-L-2’ during infection of grape leaves. Reads for Chinese isolates ‘JL-7-2’ and ‘ZJ-1-1’ come from RNA-Seq analysis of a mixed RNA sample comprised of equal amounts of total RNA extracted from infected *V. vinifera* cv. Thompson seedless leaves sampled at 24 hpi, 48 hpi and 72 hpi. In contrast, data given for the Australian isolate ‘CSIRO-L-2’ represents the highest number of RNA-Seq reads obtained from RNA sampled at any of the sampling time points of 12 hpi, 24 hpi, 48 hpi, 72 hpi or 96 hpi of *V. vinifera* cv. Cabernet sauvignon leaves. Therefore direct comparisons between the number of reads obtained for each *P. viticola* transcript cannot be made between the Chinese and Australian isolates. Predicted RXLR effectors are highlighted in yellow.

**Supplementary Table S6.** Summary of repeat elements in the *P. viticola* genome.

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**Supplementary Table S10.** Predicted pathogenicity proteins within the *P. viticola* secretome. BLAST analysis was performed with the entire *P. viticola* secretome against the Pathogen-Host Interaction Database (PHI database).

**Supplementary Table S11.** Putative carbohydrate-active enzyme (CAZyme) classes encoded in the *P. viticola* secretome as predicted using the dbCAN database.

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**Supplementary Table S13.** Protein identity comparison between all proteins and secreted proteins predicted in *P. viticola* genome.

**Supplementary Table S14.** Presence and absence of important metabolic enzymes in *P. viticola* and comparison with *Phytophthora* species, other downy mildews and *A. laibachii*. Red indicates absence and green indicates presence of genes. Genes present for all the organisms below were annotated by blast to NCBI non-redundant protein database or KEGG database and genes present in *P. viticola* were also validated by PCR and transcriptome data. The primers were listed in Supplementary Table S15.

**Supplementary Table S15.** Primers were used to validate the presence of enzymes related to nitrogen,

sulfur and thiamine metabolic pathways by PCR amplification from genomic DNA of 'JL-7-2' isolate.

**Supplementary Table S1.** Summary of sequencing data information of *P. viticola* isolate 'JL-7-2'.

Data Type	Insert Size	Raw reads	Trimmed reads	Sequence Depth
	180 bp	76,363,088	73,311,382	66.1X
Illumina	500 bp	48,061,012	44,068,164	38.6X
Paired-end	800bp	47,564,330	23,866,398	20.4X
	1kb	51,285,508	15,151,256	12.6X
Illumina	3kb	31,320,134	23,352,680	21.0x
Mate pair	6kb	12,799,230	11,471,298	10.2x
PacBio	-	2,404,672	845,976	38.0X
Total	-			207.0X

**Supplementary Table S2.** Comparison of *P. viticola* genome assembly statistics using Illumina sequence data only or Illumina/PacBio hybrid assembly.

	Illumina Only	Illumina/PacBio assembly
Genome assembly size	103,157,395	101,296,116
Number of ambiguous bp	28,824,422	16,928,280
The percentage of gaps	27.94%	16.71%
N50 of scaffold	160,129	172,266
Length of largest scaffold	692,625	805,677
Total number of scaffolds	2793	2165

**Supplementary Table S3.** Comparison of genome assembly statistics of two sequenced *P. viticola* isolates.

<i>P. viticola</i> Isolates	JL-7-2 (China, present work)	INRA-PV221 (France <sup>28</sup> )
Estimated genome size (without N)	84.37 Mb	72.74 Mb
Number of scaffolds	2,165 (>500 bp)	1,883 (>1000 bp)
Scaffold N50 size (kb)	172.3 kb	180.6 Kb
Longest scaffold (kb)	805.7	763.5
GC content of whole genome (%)	45%	44.3%
% Repeats	25.6%	29.8%
CEGMA/BUSCO (with partials)	97% /90%	95%/na

**Supplementary Table S4.** Comparison of different oomycete genome assembly by BUSCO analysis. A total of 429 conserved eukaryotes proteins were searched from the assembled *P. viticola* and other 5 oomycete genomes.

Species	Complete	Duplicated	Fragmented	Missing
<i>P. viticola</i>	84%	46%	6%	9.50%
<i>H. arabidopsidis</i>	79%	32%	8.80%	11%
<i>P. halstedii</i>	90%	24%	4.10%	5.30%
<i>P. infestans</i>	90%	29%	2%	6.90%
<i>P. sojae</i>	88%	24%	3.20%	8.30%

**Supplementary Table S5. Transcription of predicted *P. viticola* genes during infection.** Number of RNA-Seq reads detected in RNA extracted expressed genes of *P. viticola* isolates ‘JL-7-2’, ‘ZJ-1-1’ and ‘CSIRO-L-2’ during infection of grape leaves. Reads for Chinese isolates ‘JL-7-2’ and ‘ZJ-1-1’ come from RNA-Seq analysis of a mixed RNA sample comprised of equal amounts of total RNA extracted from infected *V. vinifera* cv. Thompson seedless leaves sampled at 24 hpi, 48 hpi and 72 hpi. In contrast, data given for the Australian isolate ‘CSIRO-L-2’ represents the highest number of RNA-Seq reads obtained from RNA sampled at any of the sampling time points of 12 hpi, 24 hpi, 48 hpi, 72 hpi or 96 hpi of *V. vinifera* cv. Cabernet sauvignon leaves. Therefore direct comparisons between the number of reads obtained for each *P. viticola* transcript cannot be made between the Chinese and Australian isolates. Predicted RXLR effectors are highlighted in yellow.

( See separate excel file)

**Supplementary Table S6.** Summary of repeat elements in the *P. viticola* genome.

Repeat Elements	Percentage	Number of bases
SINE	0.21%	208618
Unknown	6.55%	6631664
Simple_repeat	0.25%	253996
rRNA	0.08%	79398
RC/Helitron	0.02%	19915
LTR/Gypsy	10.39%	10531330
LTR/Copia	3.15%	3194722
Low_complexity	0.03%	29963
DNA/TcMar-Tc2	-	-
LINE/RTE-X	0.20%	206820
LINE/RTE-BovB	0.33%	336879
LINE/R2	0.02%	23845
LINE/Penelope	0.04%	40107
DNA/TcMar-Pogo	-	-
LTR/Ngaro	-	-
LINE/L1-Tx1	1.08%	1096284
LINE/L1	0.28%	283826
LINE/R1	-	-
LINE/telomeric	-	-
DNA/TcMar-Tc1	-	-
LINE/Jockey	0.11%	115846
DNA/Harbinger	-	-
DNA/TcMar-Tigger	0.03%	32269
DNA/TcMar-Fot1	0.07%	74427
DNA/TcMar-	-	-
DNA/Sola	0.14%	137774
DNA/MuLE-MuDR	0.68%	690357
DNA/Maverick	1.61%	1633176
DNA/hAT-hobo	0.02%	18627
DNA/hAT-Ac	0.09%	93893
DNA/hAT-Charlie	-	-
DNA/hAT-Tag1	-	-
DNA/hAT-Tip100	-	-
DNA/Crypton	0.15%	149261
<b>Total</b>	<b>25.6 %</b>	<b>25882997</b>

**Supplementary Table S7.** Candidate RXLR/Q effectors encoded in the *P. viticola* genome.

(See separate excel file)

**Supplementary Table S8.** Gene density and percentage of repeat elements in the *P. viticola* genome and in scaffolds with RXLR clusters.

	Gene Density (# gene per Mb)	Percentage of repeat elements
Seven scaffolds containing RXLR clusters	139	36.09%
All <i>P. viticola</i> genome scaffolds	176	25.55%

**Supplementary Table S9.** Candidate CRN effectors in the *P. viticola* genome.

(See separate excel file)

**Supplementary Table S10.** Predicted pathogenicity proteins within the *P. viticola* secretome. BLAST analysis was performed with the entire *P. viticola* secretome against the Pathogen-Host Interaction Database (PHI database).

(See separate excel file)



**Supplementary Table S11.** Putative carbohydrate-active enzyme (CAZyme) classes encoded in the *P. viticola* secretome as predicted using the dbCAN database.

	CAZy families	Number	Total number
<b>Auxiliary Activities (AAs)</b>	AA10	1	4
	AA7	1	
	AA7	1	
	AA8	1	
<b>Carbohydrate-Binding Modules (CBMs)</b>	CBM1	4	9
	CBM13	1	
	CBM15	1	
	CBM50	1	
	CBM63	1	
	CBM9	1	
<b>Carbohydrate Esterases (CEs)</b>	CE1	4	16
	CE10	2	
	CE13	1	
	CE4	2	
	CE5	1	
	CE8	6	
<b>Glycoside Hydrolases(GHs)</b>	GH102	1	60
	GH109	1	
	GH131	13	
	GH17	9	
	GH19	3	
	GH23	2	
	GH28	1	
	GH3	5	
	GH30	1	
	GH32	2	
	GH43	1	
	GH5	3	
	GH6	13	
	GH7	1	
	GH72	4	
	<b>Glycosyltransferases (GTs)</b>	GT31	
GT4		2	
GT71		1	
<b>Polysaccharide lyases (PLs)</b>	PL22	1	1

**Supplementary Table S12.** Differentially expressed genes in *P. viticola* isolate ‘ZJ-1-1’ during infection of *V. amurensis* cv. Shuanghong.

(See separate excel file)

**Supplementary Table S13.** Amino acid identity comparison between all proteins and secreted proteins predicted in *P. viticola* compared to other oomycete genomes.

>60% amino acid identity	<i>P. halstedii</i>	<i>P. infestans</i>	<i>P. sojae</i>	<i>H. arabidopsidis</i>
All proteins ( <i>P. viticola</i> )	9657 (56.8%)	8545 (50.2%)	8164(50.0%)	6035 (35.5%)
Secreted proteins ( <i>P. viticola</i> )	733 (43.5%)	698 (41.4%)	672 (39.9%)	426 (25.3%)

**Supplementary Table S14.** Presence and absence of important metabolic enzymes in *P. viticola* and comparison with *Phytophthora* species, other downy mildews and *A. laibachii*. Red indicates absence and green indicates presence of genes. Genes present for all the organisms below were annotated by blast to NCBI non-redundant protein database or KEGG database and genes present in *P. viticola* were also validated by PCR and transcriptome data. The primers were listed in Supplementary Table S14.

	<i>P. viticola</i>	<i>P. halstedii</i> <sup>1</sup>	<i>H. arabidopsidis</i> <sup>2</sup>	<i>P. sojae</i> <sup>2</sup>	<i>P. infestans</i> <sup>2</sup>	<i>A.laibachii</i> <sup>3</sup>
Nitrate reductase	Pv11323	Phal04323	none	Ps140563	PITG_13012.1	none
Nitrite reductase	none	none	none	Ps140562	PITG_13013.1	none
Nitrate transporter	Pv07654	Phal07021	none	Ps140564	PITG_13011.1	CCA24412
Sulphite oxidase	Pv02147 Pv10357	Phal16715 Phal03654	Ha812582	Ps250369	PITG_12342.1	none
Sulphite reductase	none	none	none	Ps139488 Ps139493	PITG_19263.1 PITG_18187.1	none
Cysteine synthetase	Pv02771 Pv05868	Phal08105	Ha814750	Ps109172 Ps109175	PITG_12727.1 PITG_12725.1	none
Thiamine-phosphate synthase	none	none	none	none	none	none
Thiamine pyrophosphokinase	Pv08978 Pv10004	Phal05670	Ha803592	Ps565547	PITG_07866.1	CCA24794

1 Gene IDs from the Broad Institute Database: <http://www.broadinstitute.org>.

2 Gene IDs from the VBI Microbial Database: [vmd.vbi.vt.edu](http://vmd.vbi.vt.edu).

3 Gene IDs from <http://dataportal-senckenberg.de/database/metacat/rsharma.26.2/bikf>.

**Supplementary Table S15.** Primers were used to validate the presence of enzymes related to nitrogen, sulfur and thiamine metabolic pathways by PCR amplification from genomic DNA of 'JL-7-2' isolate.

Primers	Sequences (5'to3')
Pv11323-F	GTGGAACAAGAGTGGCTGGATACGG
Pv11323-R	GTCCAAGACACTCCAGCA
Pv07654-F	ATGCCACAAAGTGCATCGTAC
Pv07654-R	TTACTCGATAGGCGCTAGTTG
Pv08978-F	TGTTATCGGACGACTA
Pv08978-R	GATGCCACCACTTCTACT
Pv10004-F	GTTACTAAAGAGGGCGAAAG
Pv10004-R	AGACGCAGTGAGTCCAAAT

**Figure S1.** Genomic distribution of contig length (N length) versus contig number (N number). N lengths were calculated by ordering all sequences according to their length and then adding the length from longest to shortest until the summed length exceeded 10% (N10), 20% (N20), etc., up to 100% (N100) of the assembled contigs. Plotting the N length versus the N number (number of contigs in each N category) indicates that 90% of the assembled genome show high continuity, while the last 10% are highly fragmented.

**Figure S2.** Identification of single copy core eukaryotic orthologous genes (CEGs) by the CEGMA pipeline. Group 1 represents the least conserved of the 248 core eukaryotic genes, with the degree of conservation increasing in subsequent groups through Group 4.

**Figure S3. Highly conserved RXLR effectors identified in multiple oomycete species.** Multiple sequence alignment of two conserved PvRXLRs (a) and one PvCRN (b) between different oomycete species. The RXLR and dEER motifs are indicated with a black boxes. The alignment was constructed using BioEdit3.3.19.0 software. The threshold (%) for shading was set at 50. Similar amino acid residues are shaded grey and identical amino acid residues are shaded black.

**Figure S4.** Molecular divergence (a) and phylogenetic relationship (b) between *P. viticola* and other species based on pairwise comparisons of the one-to-one orthologues. In the figure (a), the cumulative frequencies of amino acid identity across each set of potential orthologous pairs is presented.

Figure S1.

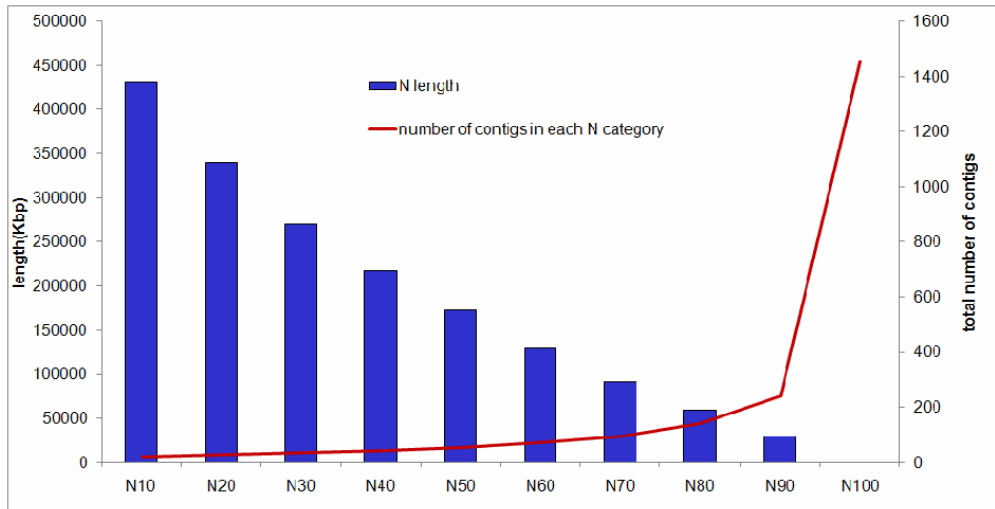


Figure S2.

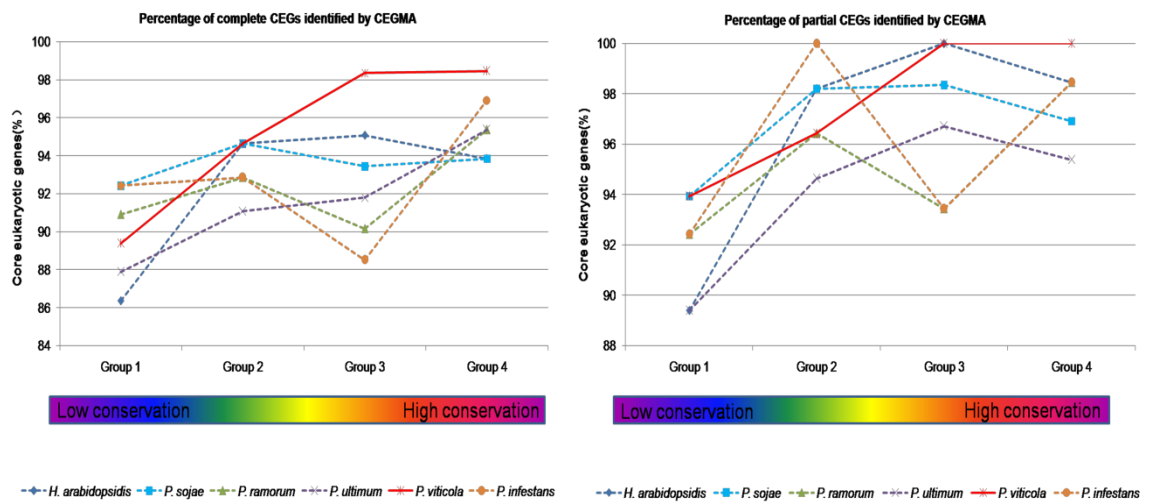


Figure S3.

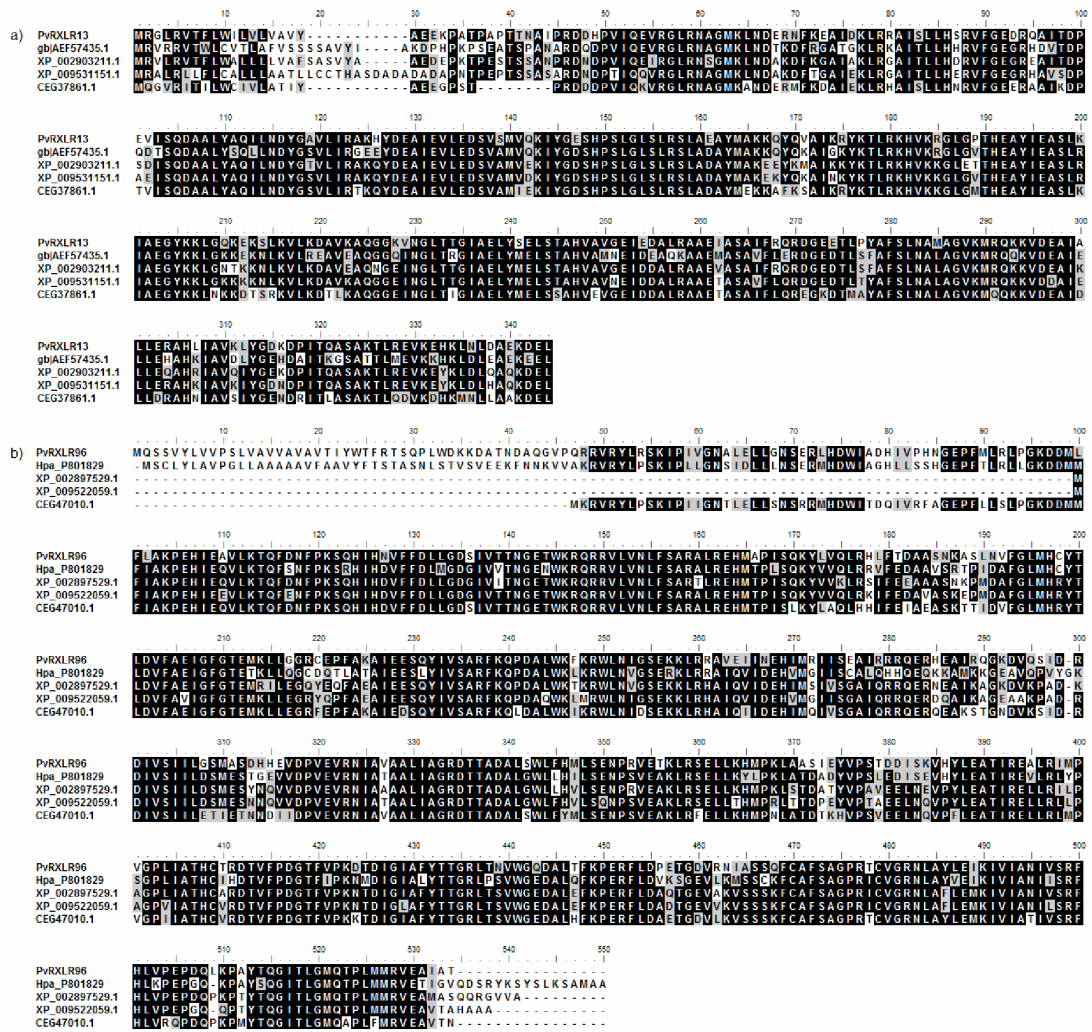


Figure S4.

