VIROLOGICA SINICA

Electronic Supplementary Material

Blue-white Colony Selection of Virus-infected Isogenic Recipients Based on a Chrysovirus Isolated from Penicillium italicum

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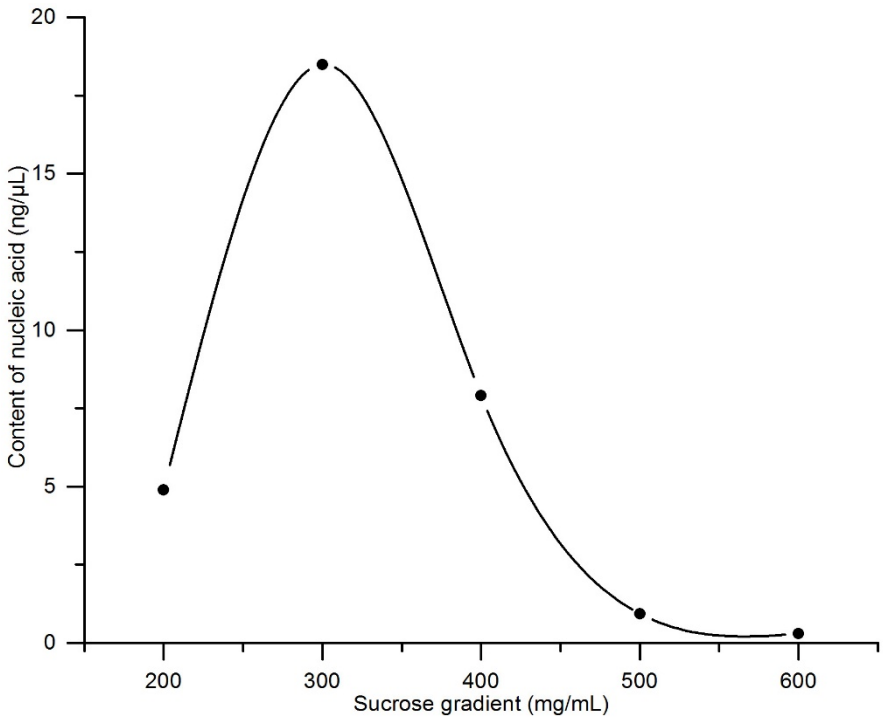

Fig. S1 Nucleic acid abundance profile of fractions separated using sucrose concentration gradients.

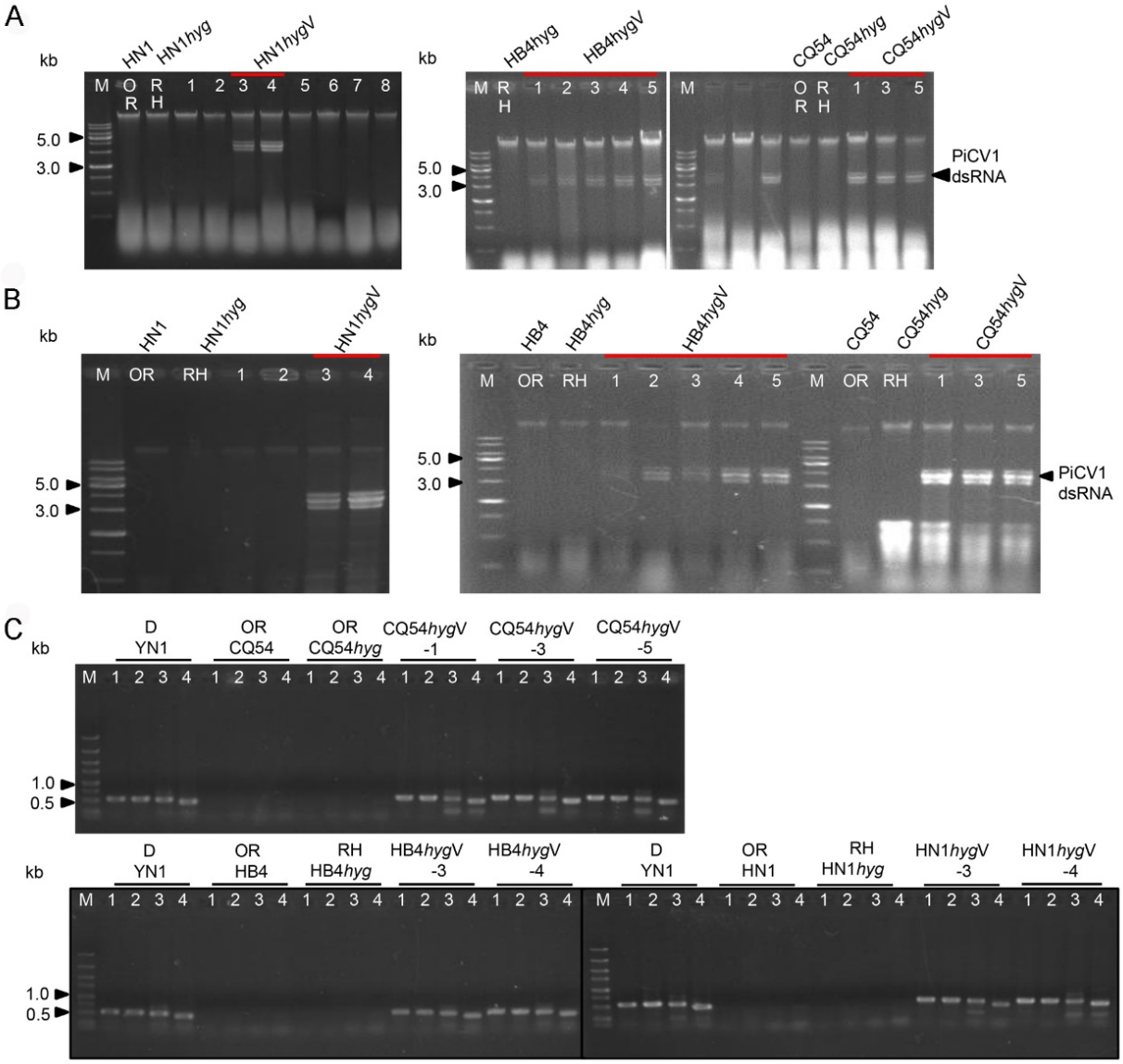

Fig. S2 Total genome detection and viral genome verification using PiCV1-infected isolates by hygromycin B screening. (A) Screening of virus-infected isolates by agarose gel electrophoresis of total DNA after hyphal anastomosis and isolation culture of single spores. (B) dsRNA detection in isolates infected with PiCV1. (C) Verification of virus-infected individuals by RT-PCR using specific probe primers targeting the four dsRNA segments of PiCV1. 1–4 indicate the PiCV1 genome dsRNA segment number. NO. of selected isolates; D: donor; OR: original recipient; RH: recipient overexpressing the selection marker *hyg*.

Table S1 Primer list

Primer name	Sequence	Objective
ITS1	TCCGTAGGTGAACCTGCGG	Internal transcribed spacer (ITS) amplification
ITS4	TCCTCCGCTTATTGATATGC	
dsRNA1 F	GAGAATAATTCCTTTGGACCTGAG	cDNA amplification and clone of dsRNA1 for Sanger sequence
dsRNA1 R	ATAGCATGCTTGAATACAGAGGC	
dsRNA1 F1	GAATAATTCCTTTGGACCTGAGG	
dsRNA1 R1	AAGAATTTGCTGCACCGGATC	
dsRNA1 F2	TGATCCGGTGCAGCAAATTCTT	
dsRNA1 R2	CGAGCCAACACGTTGACGTAG	
dsRNA2 F	TGGCTGCCCCTGTTCTAT	cDNA amplification and clone of dsRNA2 for Sanger sequence
dsRNA2 R	CAGTGATTCCTAACCATTAACC	
dsRNA2 F1	TGCCCGTGGTCTGGGATAAATG	
dsRNA2 R1	CGGGAGGTTCATCTATGATCGGT	
dsRNA2 F2	ACGTGAAGGCTTGCAATTAAC	
dsRNA3 F	AAAAAAACGATAAGCGGTAGCC	cDNA amplification and clone of dsRNA3 for Sanger sequence
dsRNA3 R	GTATGACTGTTCCCAGTCCGGT	
dsRNA3 R1	CAATTATCTAGGCTCGGTATTGC	
dsRNA3 F2	ATCAACGCGCAGGGGCTAAAG	
dsRNA3 R2	TGCAGCACTACCTTCTGATCCATC	
dsRNA4 F	AACAATAATTCCTAGCAGCCTGAG	cDNA amplification and clone of dsRNA4 for Sanger sequence
dsRNA4 R	GAATTAAGCTCATGAAGGCTCTAG	
dsRNA4 R1	ATGTGCTGTCCTATGTCCCACT	
PC3-T7 loop*	5'-p-GGATCCCGGGAATTCGGTAATACGACTCA CTATATTTTTATAGTGAGTCGTATTA-NH_2-3'	Adaptor ligated to ends of each dsRNA
PC2	CCGAATTCCCGGGATCC	Plus strand 3′-terminal determination by RACE-PCR
dsRNA1 p3′ F	AACATTTGCTGAATTTCCGC	
dsRNA2 p3′ F	TAATATACCGTCCGACGCTC	
dsRNA3 p3′ F	CCGGTGTAGATTCAGCGTTAT	
dsRNA4 p3′ F	CAGCAAAGGCTACAGAGCTG	
PC2	CCGAATTCCCGGGATCC	Minus strand 3′-terminal determination by RACE-PCR
dsRNA1 m3′ F	GGCACCGAGTAATCAAGCAGT	
dsRNA2 m3′ F	ACGGGCACCTCAGCAGTCATT	
dsRNA3 m3′ F	AGGGGTTATTAGACTGTCGC	
dsRNA4 m3′ F	CTTTGACATGATCATTCGTCGT	
dsRNA1 probe F	TAGAGTCGGCTAACTACGGGTCG	Probe1 amplification and RT-PCR detection
dsRNA1 probe R	ACCCGATCCGAGGGCTTACA	
dsRNA2 probe F	GGTCAAGTTCGGGCGACAGAT	Probe2 amplification and RT-PCR detection
dsRNA2 probe R	ACGTAGCATCGCCCAGGTAG	
dsRNA3 probe F	GCCATCTTGGGAAAGGTGAC	Probe3 amplification and RT-PCR detection
dsRNA3 probe R	GTGACTCAGTAGTATGGCTGGTTT	
dsRNA4 probe F	TGGCGTGAGTTGCCTTATGT	Probe4 amplification and RT-PCR detection
dsRNA4 probe R	GCAAGTGCCAGGGCGTATTC	
PiPksP L F	TACAATGATGCCTTCAGGTG	Amplification of upstream sequence of pksP knockout site
PiPksP L R	CGAAGACATCGTACGTACACTGACTAGTAAGAA	

	TGCTCCTACTAGCGAG	(L-arm)
Hyg cassette F	GACTAGTCAGAAGATGATATTGAAGGAGC	Amplification of hyg cassette to fuse between L-arm and R-arm; Positive PCR amplification
Hyg cassette R	AGTGTACGTAAAGAAGGATTACCTCTAAACAAG	
PiPksP R F	AGTGTACGTACGATGTCTTCGCTCCTGACTAC	Amplification of downstream sequence of pksP knockout site (R-arm)
PiPksP R R	GCGCATTGATACAAGCCACCTC	
pksP KO F	AGCCCACAAGAGTTCCAATCCAC	Negative PCR amplification
pksP KO R	TTCCATCTGCTCTGCAATAGCC	

* 5′ and 3′ side of adaptor was phosphorylated and aminated respectively.

Table S2. Information on the virus isolates used for sequence alignment and phylogenetic analysis of their RdRps.

Virus name	Abbreviation	Accession no.	Family
Botryosphaeria dothidea chrysovirus 1	BdCV1	KY111917	*Chrysoviridae*
Isaria javanica chrysovirus 1	IjCV1	KX898418	*Chrysoviridae*
Penicillium janczewskii chrysovirus 1	PjCV1	KT601116	*Chrysoviridae*
Fusarium oxysporum f. sp. dianthimycovirus 1	FoCV1	KP876630	*Chrysoviridae*
Magnaporthe oryzae chrysovirus 1-B	MoCV1-B	AB824668	*Chrysoviridae*
Magnaporthe oryzae chrysovirus 1-A	MoCV1-A	AB560762	*Chrysoviridae*
Amasya cherry disease associated chrysovirus	ACCV	AJ781167	*Chrysoviridae*
Penicillium chrysogenum virus	PcV	AF296440	*Chrysoviridae*
Penicillium italicum Chrysovirus1	PiCV1		*Chrysoviridae*
Helminthosporium victoriae 145S virus	HV 145S	AF297177	*Chrysoviridae*
Cryphonectria nitschkei chrysovirus 1	CnCV1	GQ290645	*Chrysoviridae*
Verticillium dahliae chrysovirus 1	VdCV1	HM004068	*Chrysoviridae*
Saccharomyces cerevisiae virus La	StV La	NC_001641	*Totiviridae*
Xanthophyllomyces dendrorhous virus L1A	XdV-L1A	JN997472	*Totiviridae*
Helminthosporium victoriae virus 190S	HvV190S	NC_003607	*Totiviridae*
Magnaporthe oryzae virus 1	MoV1	AB176964	*Totiviridae*
Magnaporthe oryzae virus 2	MoV2	AB300379	*Totiviridae*
Tolypocladium cylindrosporum virus 1	TcV1	FR750562	*Totiviridae*
Rosellinia necatrix victorivirus 1	RnVV1	AB742454	*Totiviridae*
Beauveria bassiana victorivirus NZL	BbVV NZL	KJ364649	*Totiviridae*
Discula destructiva virus 1	DdV1	AF316993	*Partitiviridae*
Discula destructiva virus 2	DdV2	AY033437	*Partitiviridae*
Fusarium solani virus 1	FsV1	NC_003885	*Partitiviridae*
Penicillium stoloniferum virus S	PsV S	AY156522	*Partitiviridae*
Aspergillus ochraceous virus	AoV	EU118278	*Partitiviridae*
Heterobasidion partitivirus 2	HpV2	HM565954	*Partitiviridae*
Cannabis cryptic virus	CcV	JN196537	*Partitiviridae*
Fusarium poae virus 1	FpV1	LC150607	*Partitiviridae*
Rosellinia necatrix partitivirus 6	RnPV6	LC010953	*Partitiviridae*
Ceratocystis resinifera virus 1	CrV1	AY603051	*Partitiviridae*
Vicia cryptic virus	VcV	AY751738	*Partitiviridae*
Beet cryptic virus 1	BcV1	EU489062	*Partitiviridae*
Rhizoctonia solani dsRNA virus 3	RsdV3	NC_032149	*Partitiviridae*
Heterobasidion partitivirus 1	HpV1	HQ541324	*Partitiviridae*

PS: The amino acid sequences alignment of viruses coloured yellow shading is presented in Fig. 3.