

Figure S1. Phylogenetic trees based on amino acid sequences of conserved proteins of phage vB_PmiS_PM-CJR and related bacteriophages. **(a)** Phylogenetic analysis of terminase large subunits. **(b)** Phylogenetic analysis of major capsid proteins. Multiple sequence alignments were generated by MAFFT in L-INS-I mode and trimmed with TrimAl. Phylogenetic trees were reconstructed with IQ-TREE 2 and visualized with FigTree, with bootstrap support values reported over tree branches. As the analysis was used to understand phylogenetic relationships of phages rather than reconstruct their evolutionary history, no rooting was applied.

vB_PmiS_PM-CJR/1- 1 NSDCCKNYYSEBDIKALKESELHIEBVARSRNLAGEKVLSTVDTIRGKTYTNTLQDGLBLYQDALSNIYQQMGGDEPGQINITGRQIVFENGWYIYRGDLPHVTGASLAEDGGVSEENPNQGV VNVQ QACIKEE-----LASSNGAN 146
 vB_PmiP_RS10pmA/1 1 NSDCCKNYYSEBDIKALKESELHIEBVARSRNLAGEKVLSTVDTIRGKTYTNTLQDGLBLYQDALSNIYQQMGGDEPGQINITGRQIVFENGWYIYRGDLPHVTGASLAEDGGVSEENPNQGV VNV-----NYDNFS 136
 vB_PmiS-Isfahan/1 1 NSDCCKNYYSEBDIKALKESELHIEBVARSRNLAGEKVLSTVDTIRGKTYTNTLQDGLBLYQDALSNIYQQMGGDEPGQINITGRQIVFENGWYIYRGDLPHVTGASLAEDGGVSEENPNQGV VNVQ YASVAEELNAKLEAPKALLASNDGSS 157
 2207-N35/1-665 1 NSDCCKNYYSEBDIKALKESELHIEBVARSRNLAGEKVLSTVDTIRGKTYTNTLQDGLBLYQDALSNIYQQMGGDEPGQINITGRQIVFENGWYIYRGDLPHVTGASLAEDGGVSEENPNQGV VLL-----TYNSYD 136

vB_PmiS_PM-CJR/1- 1 KSDGCIADDSNAI1QKALDYCEKKVGLTKGYGLSKSVYKTIYLPP----MLLPTESEV KKVFISGKSGNDW-VSSMEDPIMSQNIS 272
 vB_PmiP_RS10pmA/1 137 NQR-----IFKKIGQKIQLTDPSILDPDGVVYVDDESKDSSCILBAI-----OKESTLYFP-----GVYRVDKTIESKPGGLIPFDKATLIRLKYSSTSVPF-----SLRQVS-----B 236
 vB_PmiS-Isfahan/1 158 HIGP-----S-EGINLKEERLTQWFNASDYG---LILSKNTPENKSEALRKLS-----ADVKLNGGV-----IIPGGE-----YLIQBSRNSP--ADHPDNIRAYGV K 241
 2207-N35/1-665 137 KYK-----RTISLDYFVN-----DGDNFLQKAQSDWCENRYALVIPKGKTYQYSERVEFBDITLLWEGTL-ELIQPIDGGLVFRRNVTMPTGYLTKSTKTVLPDDY-----YMWGFYSWKSPDGSLGCQDNIS--G 257

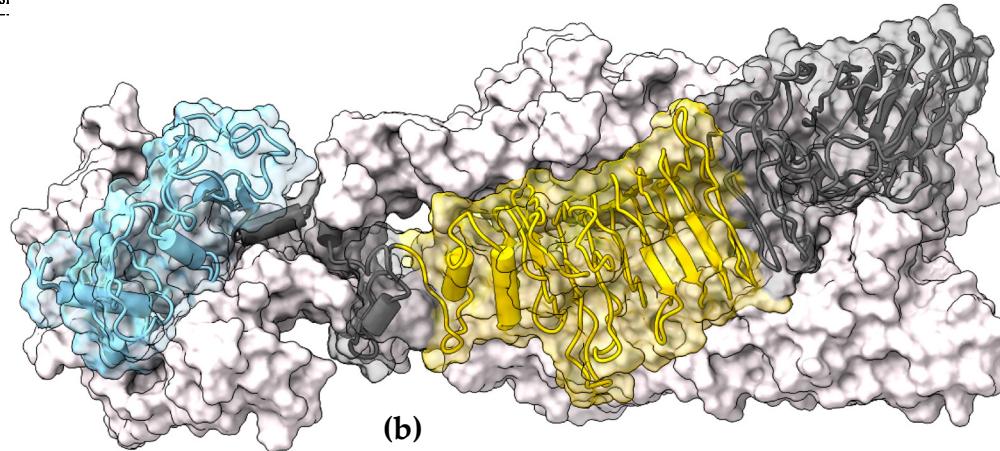
vB_PmiS_PM-CJR/1- 273 NLTVRNLLIN-----NDDVYDSYC---RYAFGFK-----ELHINCKTE-----NLJISRNFELGFDFNNCFLYLHSLLNNIVL-----NSEPLL-----KTSTGHKYRNGCNL-MKI EAAIALNTKDGYVFEAGYTCVYLDKWSFEG 387
 vB_PmiP_RS10pmA/1 237 SIGGRPITENNNHPPF-GVVTGCHNSDQDSKN-----ALCWRLI-----RPIYKGVEIGNIGLMIPSQINIGTSAN-YFGYIDNPVIQ-----NADELIVFNEIANGHC-----IISPQLYKGI-----TSG 342
 vB_PmiS-Isfahan/1 242 D-LKIEG-----NDLTIMYAKLKLKDGMKGAFDPETGEPRRPSKALFIDTECIRNRI-NIMGNVAGGYVVL-----GGQYGHYGRQCDGYGMRI RNYKSLSIKDVTINKWPLDGL-----YTG 368
 2207-N35/1-665 268 RLDIRNGHIDYDGWDQGVN-----DDPANLTGGVGLMGGSWL-----RPSLCGVDESSWENV-----HRNDLKFYYD-CRSYA-----LTLLQGIYDSESVNEIF-----GWVNGNT-----IDLARRA RDYISCLATSNGNTRGGD-----VS 376

vB_PmiS_PM-CJR/1- 388 QCVSIIISKGHEIFDMTIQNSYLENVGDDRDICFDFQSYSHVTFSNNNMWAKRAKHLVHKPLQPRNUIIEIQQKNPIYELPVENYFIDKGU-----YSEIHLKNTQASP-DLSNTLF-----RMKDMGQGVILHNKAINLDGLGRPTHIST V 527
 vB_PmiP_RS10pmA/1 343 ISFYG-----AYGNHVVGGFLHTS-----DNGVIGINLKRNRFENTHSSNNNG-----IEGFSVPEGGDRSKALFIDTECIRNRI-NIMGNVAGGYVVL-----NNKNDYDTYIJKSSFNNN-----I 444
 vB_PmiS-Isfahan/1 369 DGWYANQYKSPKPTILNNVKIVGCGRNWSITGNGFYS-----SNGLMSSEAAGHEVSSNPASNINV-BTEACK-----VVAVUTDMMTISNC-----AMTUTSUVNCT-UUUNCFYUWVYD-----WDTI 501
 2207-N35/1-665 377 SNVIIAELQSSKTFRRFLYCEG-ESNNFLVKWGDIAK-----NAGDILPEFSR-----CGKPAHAYE-AT-----

vB_PmiS_PM-CJR/1- 528 V-NEFIPGNYSGK-----YDNGYKSESMYGLYDP-----VKDTVNG:
 vB_PmiP_RS10pmA/1 445 R-ELKVFDKIVQNKSQFFQSQQSKKDVP-----NKKELP-----FRIEIPNSG:
 vB_PmiS-Isfahan/1 502 R-NSRILNQRGKGKSSSSLPIPKVSECLITNIN-----IDNSVPTEWVALNSDKNHLFITCTIHKNPITGI
 2207-N35/1-665 510 RPVKVTFVKRNG-----IDITGBIS-----NINGMPDGWNSAATDITVNTLDD-----VISIELHMENTP-----

vB_PmiS_PM-CJR/1- 624 IKITGJSKGNF-----NLG:
 vB_PmiP_RS10pmA/1 536 -DISYSTNGNTI-LEIYGTSRNNGNTNTIYKNSL-----SLTVTSNDESLLNVG:
 vB_PmiS-Isfahan/1 614 -LISPIKDKGKRYGIDPSGTYDVGSSTILTNRKIN1LPETCPVWKSVAIGSDKELPBNNTFYKNEYIITPSI
 2207-N35/1-665 624 -FSRPNKGDLQIGELFAKCESHN-----TRGVIG:

(a)



(b)

Figure S2. Bioinformatics analysis of the tail spike protein of vB_PmiS_PM-CJR. (a) Multiple sequence alignment of the tail spike protein sequences of vB_PmiS_PM-CJR and related *Gorganviruses*. The multiple alignment was constructed using MAFFT in L-INS-I mode and visualized with Jalview. Amino acid residues are colored by percentage identity. Conserved N-terminal region of tail spike proteins is indicated with a cyan box, amino acid residues of the catalytic pectin/pectate lyase fold domain (based on InterProScan prediction) are colored yellow. (b) Structural model of the vB_PmiS_PM-CJR tail spike based on an I-TASSER prediction. The trimeric model was assembled in UCSF ChimeraX. Conserved N-terminal region is shown in light blue, catalytic domain is shown in yellow.

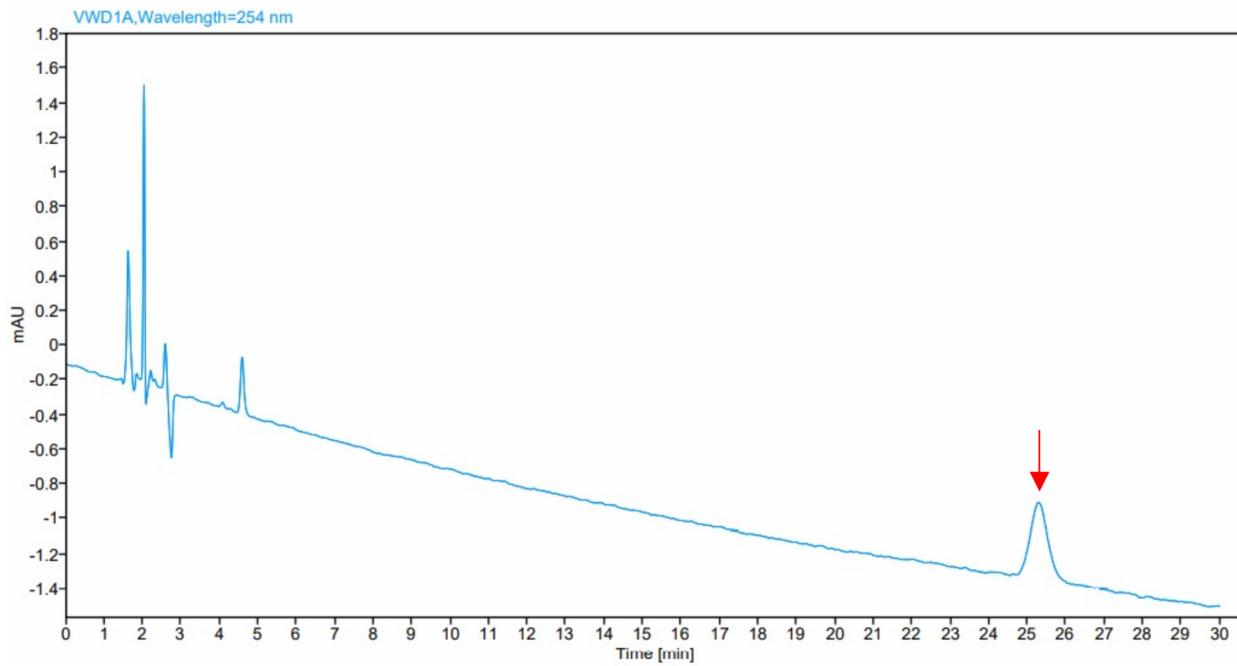


Figure S3. Purified tail spike purity analysis using reverse phase HPLC. A single peak (indicated with a red arrow) is visible at 25–26 min time mark.