

Title	Novel N4-like bacteriophages of pectobacterium atrosepticum
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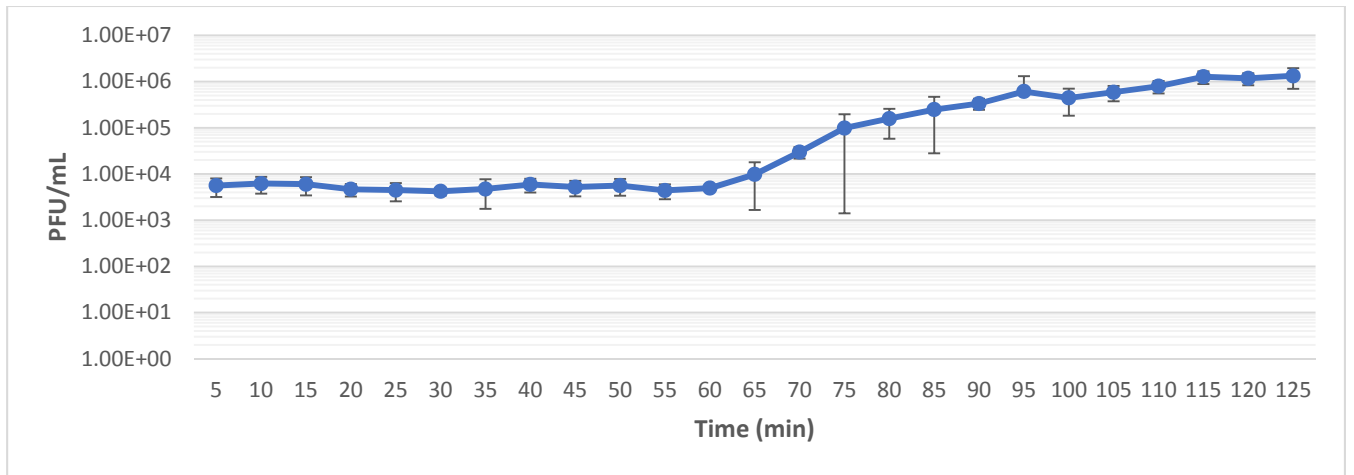
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Table S1. Results of physiological, biochemical, *Pectobacterium* genus (*pel* gene) specific and *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *carotovorum* species specific PCRs and MALDI-TOF mass spectrometry on isolates obtained from potato stem samples symptomatic for blackleg from farms in Co. Cork, Ireland.

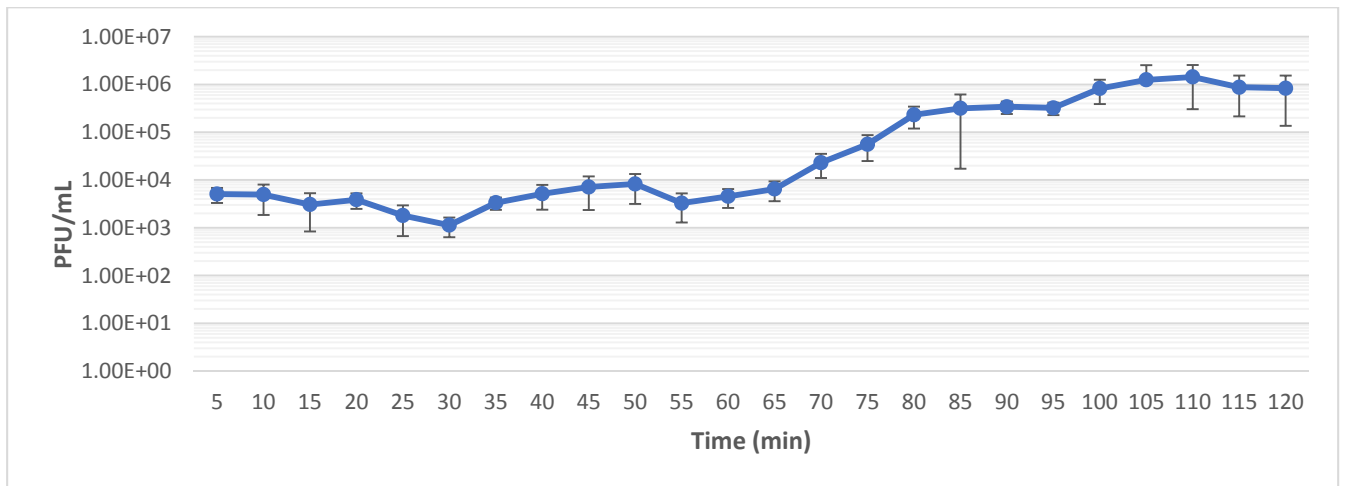
Isolate	Isolation source	Cavity formation on CVP medium (25 °C, 48 hrs)	Growth on NA at 37°C, 48 hrs	Production of reducing substance from sucrose	Acid production of reducing substance from sucrose	PCR - <i>Pel</i> gene (Darasse et al 1994)	PCR - <i>P. atrosepticum</i> (De Boer & Ward 1995)	PCR - <i>P. carotovorum</i> subsp. <i>carotovorum</i> (Kang et al 2003)	MALD-TOF MS	Identity
CB BL1-1	<i>Solanum tuberosum</i> cv. British Queen	+	-	+	+	+	+	NA	Pa	Pa
CB BL2-1	<i>Solanum tuberosum</i> cv. British Queen	+	-	+	+	+	+	NA	Pa	Pa
CB BL3-1	<i>Solanum tuberosum</i> cv. British Queen	+	-	+	+	+	+	NA	Pa	Pa
CB BL4-1	<i>Solanum tuberosum</i> cv. British Queen	+	-	+	+	+	+	NA	Pa	Pa
CB BL5-1	<i>Solanum tuberosum</i> cv. British Queen	+	-	+	+	+	+	NA	Pa	Pa
CB BL7-1	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL9-1	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL11-1	<i>Solanum tuberosum</i> cv. Rooster	+	-	+	+	+	+	NA	Pa	Pa
CB BL12-2	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL13-1	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL14-1	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL15-1	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL16-1	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL18-1	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL19-1	<i>Solanum tuberosum</i> cv. Golden wonder	+	-	+	+	+	+	NA	Pa	Pa
CB BL19-1-37	<i>Solanum tuberosum</i> cv. Golden wonder	+	+	-	-	+	-	+	NA	Pcc

Results recorded as +, positive; -, negative; NA, not available; Pa, *P. atrosepticum*; Pcc, *P. carotovorum* subsp. *carotovorum*

(A)



(B)



(C)

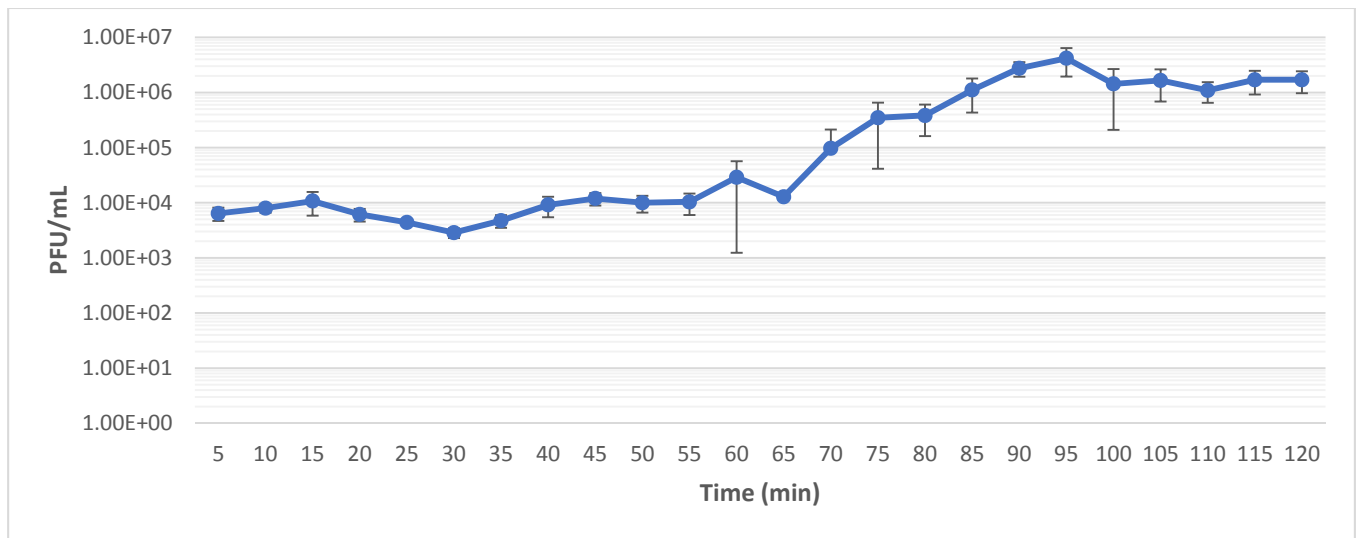


Figure S1. Single step growth curve growth analysis of phage CB1 infection of *P. atrosepticum* strain DSM18077 (A), phage CB3 infection of *P. atrosepticum* strain DSM30186 (B) and phage CB4 infection of *P. atrosepticum* DSM30186 (C). Each assay was independently repeated in triplicate and the results were averaged.

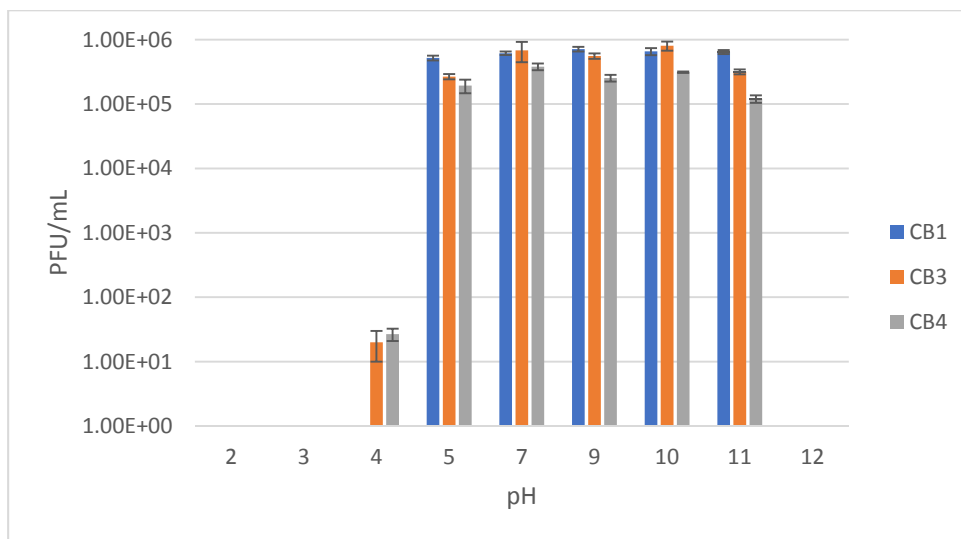


Figure S2. Stability of *Pectobacterium* phages CB1, CB3 and CB4 to various pH values upon 24 hours of exposure. Error bars represent standard deviation of biological repeats (n=3).

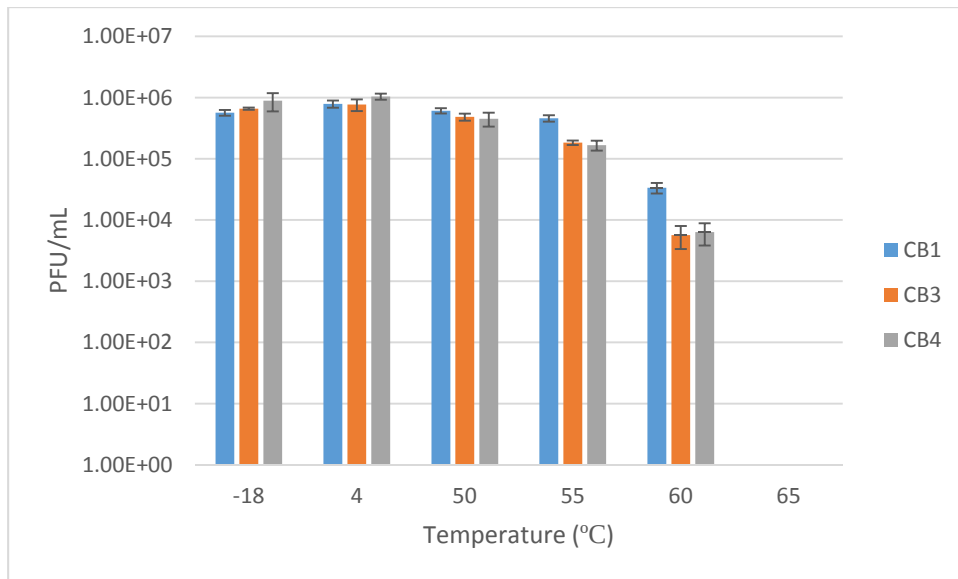


Figure S3. Stability of *Pectobacterium* phages CB1, CB3 and CB4 to various temperatures upon one-hour exposures. Error bars represent standard deviation of biological repeats (n=3).

Table S2. Identified ORFs and tRNA gene variations between the genomes of *Pectobacterium* phages CB1, CB3 and CB4. With genome comparisons made using BLASTN with ACTs. Colour coded: Green (shared feature), Yellow (unique feature to phage in question).

CB1	CB3	CB4	Variation discription
CB1_4 (hypothetical)			CB1_4 has no homolog in CB3 + CB4
CB1_8 (hypothetical)			CB1_8 has no homolog in CB3 + CB4
CB1_10 (hypothetical)	CB3_9 (hypothetical)	CB4_9 (hypothetical)	ORFs of CB1 are not share with CB3 + CB4
CB1_11 (hypothetical)	CB3_10 (hypothetical)	CB4_10 (hypothetical)	
	CB3_11 (hypothetical)	CB4_11 (hypothetical)	
	CB3_17 (hypothetical)	CB4_17 (hypothetical)	CB3/CB4_17 homolog not present in CB1
CB1_21 (hypothetical)	CB3_22 (hypothetical)	CB4_22 (hypothetical)	ORF of CB1 are not shared with CB3 + CB4
	CB3_23 (hypothetical)	CB4_23 (hypothetical)	
CB1_24 (HNH)	CB3_26 (hypothetical)	CB4_26 (hypothetical)	ORFs of CB1 are not shared with CB3 + CB4
	CB3_27 (hypothetical)	CB4_27 (hypothetical)	
CB1_26 (hypothetical)	CB3_29 (hypothetical)	CB4_29 (hypothetical)	ORF of CB1 different in part to CB3 + CB4
CB1_39 (HNH)			CB1 has no homolog in CB3 + CB4
CB1_42 (N4 gp32 like)	CB3_45 (N4 gp32 like)	CB4_44 (N4 gp32 like)	N4 gp32-like gene of CB3 different to CB1+CB4
CB1_45 (rIIB)	CB3_49 (rIIB)	ORF48 (rIIB)	Difference of CB1 ORF length compared to CB3 + CB4
CB1_61 (tail spike)	CB3_66 (tail spike)	CB4_64, 65 (tail spike)	CB4 homolog split into two ORFs
CB1_63 (thymidylate synthase)	CB3_68 (thymidylate synthase)	ORF67 (thymidylate synthase)	ORF of CB1 and CB3 different to CB4
		2 tRNA genes (tRNA1 and tRNA2)	CB4 possess tRNA genes not present in CB1 + CB3

Table S3. Homologs of the eighteen core proteins described by Li et al. 2016 found present in the genomes of *Pectobacterium* phages CB1, CB3 and CB4.

No.	N4-like Core genes	CB1 homolog	CB3 homolog	CB4 homolog
1	RNA P1	CB1_22	CB3_24	CB4_24
2	RNA P2	CB1_23	CB3_25	CB4_25
3	gp24 N4	CB1_35	CB3_38	CB4_38
4	gp25 N4	CB1_38	CB3_41	CB4_41
5	DNA P/ gp39 N4	CB1_46	CB3_50	CB4_49
6	gp42 N4	CB1_66	CB3_71	CB4_70
7	DNA primase	CB1_67	CB3_72	CB4_71
8	gp44 N4	CB1_70	CB3_75	CB4_74
9	SSB/gp45 N4	CB1_72	CB3_77	CB4_76
10	vRNAP	CB1_77	CB3_82	CB4_81
11	gp53 N4	CB1_80	CB3_85	CB4_84
12	gp54 N4	CB1_81	CB3_86	CB4_85
13	gp55 N4	CB1_82	CB3_87	CB4_86
14	MCP/ gp56 N4	CB1_83	CB3_88	CB4_87
15	gp57 N4	CB1_84	CB3_89	CB4_88
16	94kDa protein/ gp59 N4	CB1_86	CB3_91	CB4_90
17	terminase A	CB1_91	CB3_97	CB4_95
18	gp69	CB1_93	CB3_99	CB4_97

Table S4. Putative single-stranded hairpin promoters predicted in the genomes of *Pectobacterium* phages CB1, CB3 and CB4 identified assisted with QuikFold.

Phage	Promotor	Coordinates	Sequence
CB1	Porf1_1	76 - 105 bp	GTGTTGA <u>ACCGGTATCCGGT</u> ACAGTACCGT
	Porf1_2	823 - 852 bp	ATGGCAT <u>CCATGCATCATGGC</u> ATCCATATG
	Porf1_5	2085 - 2114 bp	TTGGACCGAGCGTAT <u>CGCTCAGCCC</u> ACTTA
CB3	Porf3_1	130 - 159 bp	GTGTTGA <u>ACCGGTATCCGGT</u> ACAGTACCGT
	Porf3_2	919 - 948 bp	ATGGCAT <u>CCATGCATCATGGC</u> ATCCATATG
	Porf3_4	1850 - 1879 bp	TCGGACCGCAGTAT <u>CGTGCAGCCC</u> ACTTA
CB4	Porf4_1	76 - 105 bp	GTGTTGA <u>ACCGGTATCCGGT</u> ACAGTACCGT
	Porf4_2	866 - 895 bp	ATGGCAT <u>CCATGCATCATGGC</u> ATCCATATG
	Porf4_4	1797 - 1826 bp	TCGGACCGCAGTAT <u>CGTGCAGCCC</u> ACTTA

Table S5. High ΔG rho-independent terminators predicted in the genome *Pectobacterium* phage vB_PatP_CB1 identified using ARNold and QuikFold.

Terminator	Coordinates	Sequence	ΔG kcal/mol
Torf1_1	397 - 428	GCCTACTCTTCGGAGTAGGCTTATTCTTTTCT	-17.2
Torf1_7	2,906 - 2,935	TAACCCCTTCGGGGGTTATCTTATTTTTTA	-13.6
Torf1_20	7,127 - 7,158	GCCATCCCTTCGGGGATGGCTGTTTTATTGAG	-19.3
Torf1_26	11,034 - 11,070	CGGACTCCCTAAGATGGGGAGTCCGTATTTTTTCATA	-19
Torf1_45	25,131 - 25,163	GCCAGCCCTTCGGGGCTGGTTTTTAATATCAT	-18.8
Torf1_47	28,088 - 28,113	GCCCCTTCGGGGGCTTTTTTGAGGCT	-13.7
Torf1_51	29,497 - 29,525	AGCCCCTAACGGGGCTTTTTTATTGAGGT	-12.8
Torf1_56	31,765 - 31,793	GCCCACCTAGTGTGGGCTTATATTAATCT	-10.7
Torf1_57	31,751 - 31,782, complement	AGCCCACACTAGGTGGGCTTTTTTATAGCATC	-11.8
Torf1_61	33,531 - 33,561, complement	GGGAGCCTAATGGCTCCCTTTTTAATCTGGA	-15.4
Torf1_72	45,051 - 45,078	GCCCACTTCGGTGGGCTTTTTTATCTAT	-14.7
Torf1_76	46,958 - 46,987	GCCCCTCGATTGAGGGGCTTTATTTTTTAG	-14.5
Torf1_77	46,944 - 46,976, complement	AGCCCCTCAATCGAGGGGCTTTTTATTACGGTA	-15.4
Torf1_81	62,438 - 62,467, complement	GGGAGCTTAAGGCTCCCTTTTCATTGTGAG	-11.9
Torf1_83	63,896 - 63,928, complement	GCCGGGGATAATTCCCCGGCTTTTTTATATCTA	-18.1
Torf1_87	69,766 - 69,796	CTCCCTCTTCAGAGGGAGcTTTAAACCTGA	-12.1
Torf1_88	70,620 - 70,649	GCCCCACTTCGGTGGGGCTTTTTCCGTTAT	-18
Torf1_89	71,047 - 71,076	GCCCCCGATTAAGGGGGCTTTTTTATTACA	-14.6

Table S6. High ΔG rho-independent terminators predicted in the genome *Pectobacterium* phage vB_PatP_CB3 identified using ARNold and QuikFold.

Terminator	Coordinates	Sequence	ΔG kcal/mol
Torf3_1	451 - 482	GCCTACTCTTCGGAGTAGGCTTATTCTTTTCT	-17.2
Torf3_7	2,670 - 2,699	TAACCCCTTCGGGGGTTATCTTATTTTTTA	-13.6
Torf3_21	7,283 - 7,314	GCCATCCCTTCGGGGATGGCTGTTTTATTGAG	-19.3
Torf3_29	11,386 - 11,422	CGGACTCCCCATCTTAGGGAGTCCGTATTTTTTCAT A	-17.1
Torf3_57	20,329 - 20,357	GCCAGATTTAATCTGGCTTTTTCTTTTTTA	-9.4
Torf3_49	25,440 - 25,472	GCCAGCCCTTCGGGGGCTGGTTTTTTAATATCAT	-18.8
Torf3_51	28,397 - 28,422	GCCCCTTCGGGGGCTTTTTTGAGGCT	-13.7
Torf3_55	29,806 - 29,834	AGCCCTAACGGGGCTTTTTTATTGAGGT	-12.8
Torf3_60	32,074 - 32,102	GCCACCTAGTGTTGGGCTTATATTAATCT	-10.7
Torf3_65	33,840 - 33,870, complement	GGGAGCCTAATGGCTCCCTTTTAAATCTGGA	-15.4
Torf3_77	45,360 - 45,387	GCCCACTTCGGTGGGCTTTTTTATCTAT	-14.7
Torf3_81	47,267 - 47,296	GCCCCTCGATTGAGGGGCTTTATTTTTTAG	-14.5
Torf3_82	47,253 - 47,285, complement	AGCCCTCAATCGAGGGGCTTTTTATTACGGTA	-15.4
Torf3_86	62,747 - 62,776, complement	GGGAGCTTAAGGCTCCCTTTTCATTGTGAG	-11.9
Torf3_88	64,205 - 64,237, complement	GCCGGGGATAATTCCCCGGCTTTTTTATATCTA	-18.4
Torf3_92	69,948 - 69,978	CTCCCTCTCAGAGGGAGcTTTAAACCTGA	-12.1
Torf3_94	70,802 - 70,831	GCCCCACTTCGGTGGGGCTTTTTCCGTTAT	-18
Torf3_95	71,229 - 71,258	GCCCCGATTAAGGGGGGCTTTTTTATTACA	-14.6

Table S7. High ΔG rho-independent terminators predicted in the genome *Pectobacterium* phage vB_PatP_CB4 identified using ARNold and QuikFold.

Terminator	Coordinates	Sequence	ΔG kcal/mol
Torf4_1	396 - 429	GCCTCATCCTTCGGGATGGGGCTATCTCTTTTCT	-19.6
Torf4_7	2,617 - 2,646	TAACCCCTTCGGGGGTTATCTTATTTTTTA	-13.6
Torf4_21	7,230 - 7,261	GCCATCCCTTCGGGGATGGCTGTTTTATTGAG	-19.3
Torf4_29	11,333 - 11,369	CGGACTCCCCATCTTAGGGAGTCCGTATTTTTTCAT A	-17.1
Torf4_45	20,116 - 20,144	GCCAGATTTAATCTGGCTTTTCTTTTAA	-9.4
Torf4_48	25,226 - 25,258	GCCAGCCCTTCGGGGCTGGTTTTTTAATATCAT	-18.8
Torf4_50	28,183 - 28,208	GCCCCCTTCGGGGGCTTTTTTGAGGCT	-13.7
Torf4_54	29,592 - 29,620	AGCCCCTAACGGGGCTTTTTTATTGAGGT	-12.8
Torf4_59	31,860 - 31,888	GCCCACCTAGTGTGGGCTTATATTAATCT	-10.7
Torf4_64	33,826 - 33,656, complement	GGGAGCCTAATGGCTCCCTTTTAAATCTGGA	-15.4
Torf4_76	45,615 - 45,642	GCCCACTTCGGTGGGCTTTTTTATCTAT	-14.7
Torf4_81	47,508 - 47,540, complement	AGCCCCTCAATCGAGGGGCTTTTTATTACGGTA	-15.4
Torf4_80	47,522 - 47,551	GCCCCTCGATTGAGGGGCTTTATTTTTTAG	-14.5
Torf4_85	63,002 - 63,031, complement	GGGAGCTTAAGGCTCCCTTTTCATTGTGAG	-18.1
Torf4_91	70,203 - 70,233	CTCCCTCTTCGGAGGGAGcTTTTAAACCTGA	-15.2
Torf4_92	71,175 - 71,204	GCCCCACTTCGGTGGGGCTTTTTCCGTTAT	-18
Torf4_93	71,602 - 71,631	GCCCCGATTAAGGGGGCTTTTTTATTACA	-14.6

Table S8. Shared high ΔG putative rho-independent terminators among *Pectobacterium* phages CB1, CB3 and CB4

no.	CB1	CB3	CB4
1	Torf1_01	Torf3_01	Torf4_01
2	Torf1_07	Torf3_07	Torf4_07
3	Torf1_20	Torf3_21	Torf4_21
4	Torf1_26	Torf3_29	Torf4_29
5	Torf1_45	Torf3_49	Torf4_48
6	Torf1_47	Torf3_51	Torf4_50
7	Torf1_51	Torf3_55	Torf4_54
8	Torf1_56	Torf3_60	Torf4_59
9	Torf1_61	Torf3_65	Torf4_64
10	Torf1_72	Torf3_77	Torf4_76
11	Torf1_76	Torf3_81	Torf4_80
12	Torf1_77	Torf3_82	Torf4_81
13	Torf1_81	Torf3_86	Torf4_85
14	Torf1_87	Torf3_92	Torf4_91
15	Torf1_88	Torf3_94	Torf4_92
16	Torf1_89	Torf3_95	Torf4_93

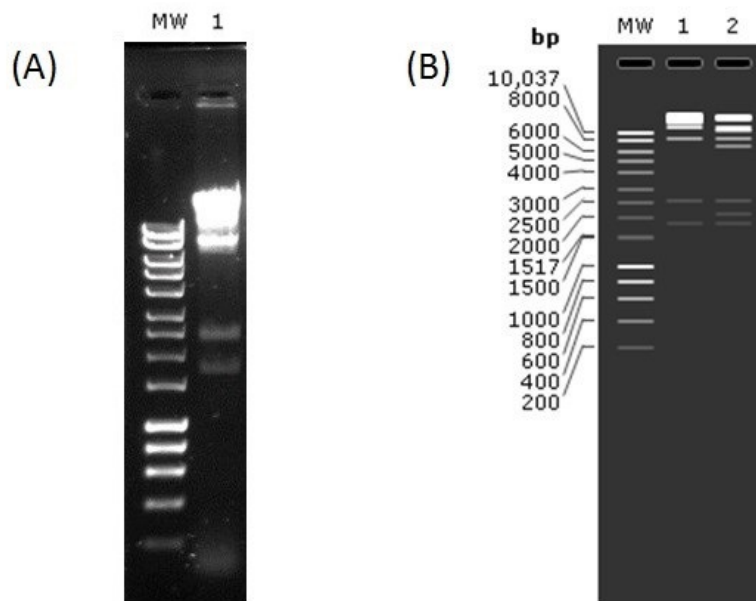


Figure S4. (A) Genomic DNA of *Pectobacterium* phage CB1, which had been digested with restriction enzyme Clal (lane 1), with DNA marker (Hyperladder 1kb, Bioline) (lane MW). (B) *In silico* digest of CB1 redundant genomic DNA with Clal with Dam methylation (lane 2); non-Dam methylation (lane 3); DNA marker (Hyperladder 1kb, Bioline) (lane MW). Gel concentration 1 % w/v agarose. Image B was generated using Snapgene.

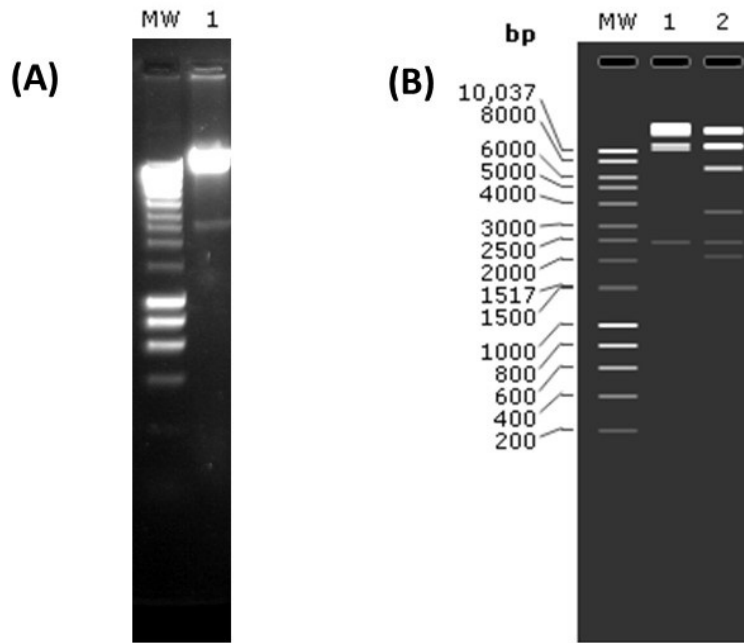


Figure S5. (A) Genomic DNA of *Pectobacterium* phage CB3, which had been digested with restriction enzyme Clal (lane 1), with DNA marker (Hyperladder 1kb, Bioline) (lane MW). (B) *In silico* digest of CB3 redundant genomic DNA with Clal with Dam methylation (lane 2); non-Dam methylation (lane 3); DNA marker (Hyperladder 1kb, Bioline) (lane MW). Gel concentration 1 % w/v agarose. Image B was generated using Snapgene.

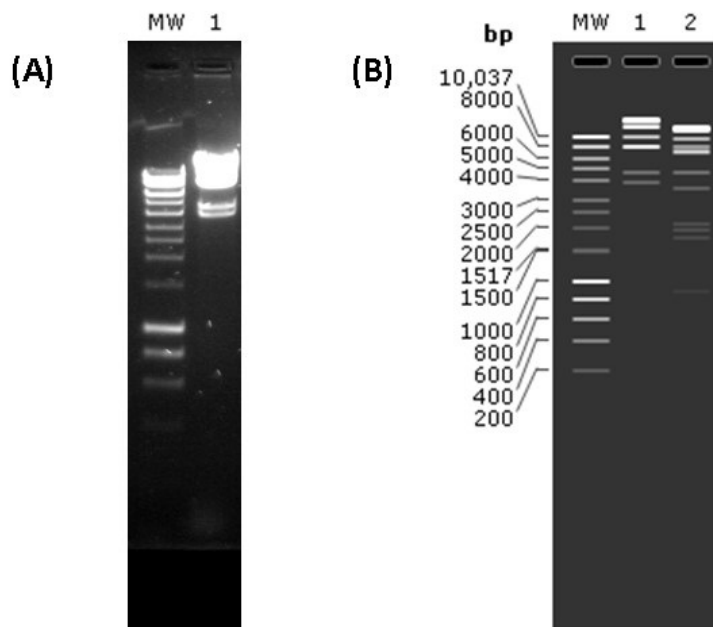


Figure S6. (A) Genomic DNA of *Pectobacterium* phage CB4, which had been digested with restriction enzyme Clal (lane 1), with DNA marker (Hyperladder 1kb, Bioline) (lane MW). (B) *In silico* digest of CB4 redundant genomic DNA with Clal with Dam methylation (lane 2); non-Dam methylation (lane 3); DNA marker (Hyperladder 1kb, Bioline) (lane MW). Gel concentration 1 % w/v agarose. Image B was generated using Snapgene.

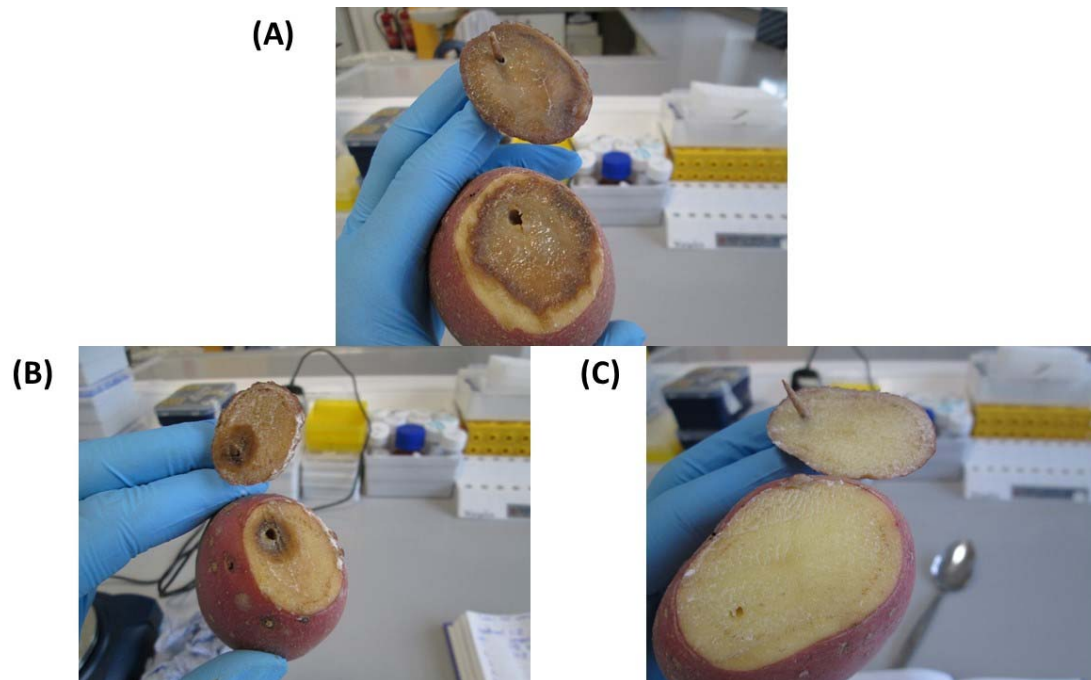


Figure S7. Pictures of the typical observed outcomes for the tuber rot assays. A: tuber treated with bacterial strains DSM 18077 + DSM 30186 and SM buffer. B: tuber treated with bacterial strains DSM 18077 + DSM 30186 and phage mixture (CB1 + CB3 + CB4). C: tuber treated with water and SM buffer.