

| | |
|-----------------------------|--|
| Title | Novel N4-like bacteriophages of pectobacterium atrosepticum |
| Authors | Buttimer, Colin;Hendrix, Hanne;Lucid, Alan;Neve, Horst;Noben, Jean-Paul;Franz, Charles M. A. P.;O'Mahony, Jim;Lavigne, Rob;Coffey, Aidan |
| Publication date | 2018 |
| Original Citation | Buttimer, C., Hendrix, H., Lucid, A., Neve, H., Noben, J.-P., Franz, C., O'Mahony, J., Lavigne, R. and Coffey, A. (2018) 'Novel N4-like bacteriophages of pectobacterium atrosepticum', <i>Pharmaceuticals</i> , 11(2), 45 [22pp]. doi: 10.3390/ph11020045 |
| Type of publication | Article (peer-reviewed) |
| Link to publisher's version | http://www.mdpi.com/1424-8247/11/2/45 - 10.3390/ph11020045 |
| Rights | © 2018, the Authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). - http://creativecommons.org/licenses/by/4.0/ |
| Download date | 2023-10-02 12:46:08 |
| Item downloaded from | https://hdl.handle.net/10468/6919 |



UCC

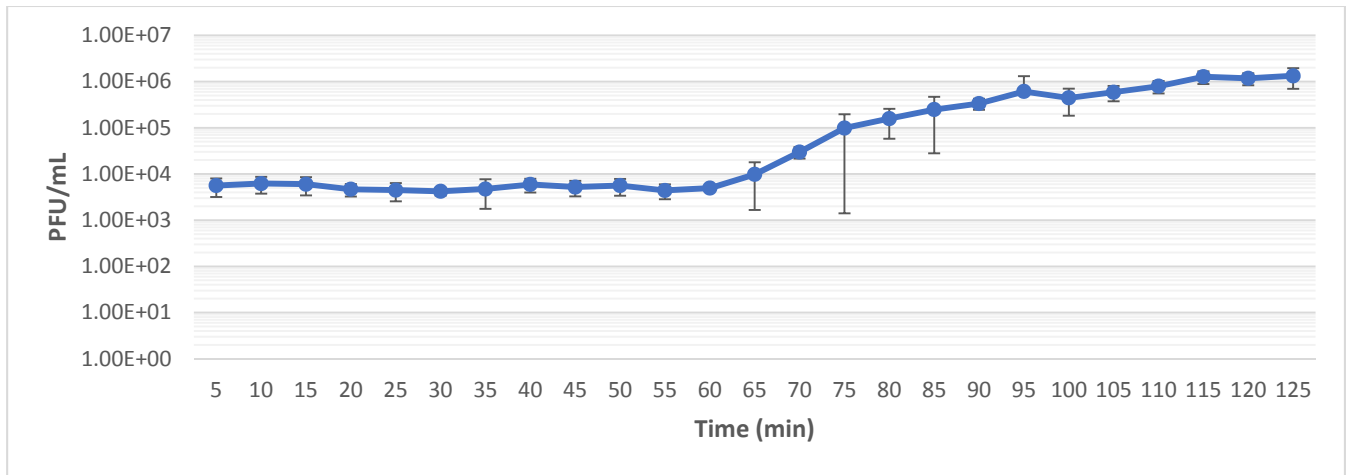
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

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.

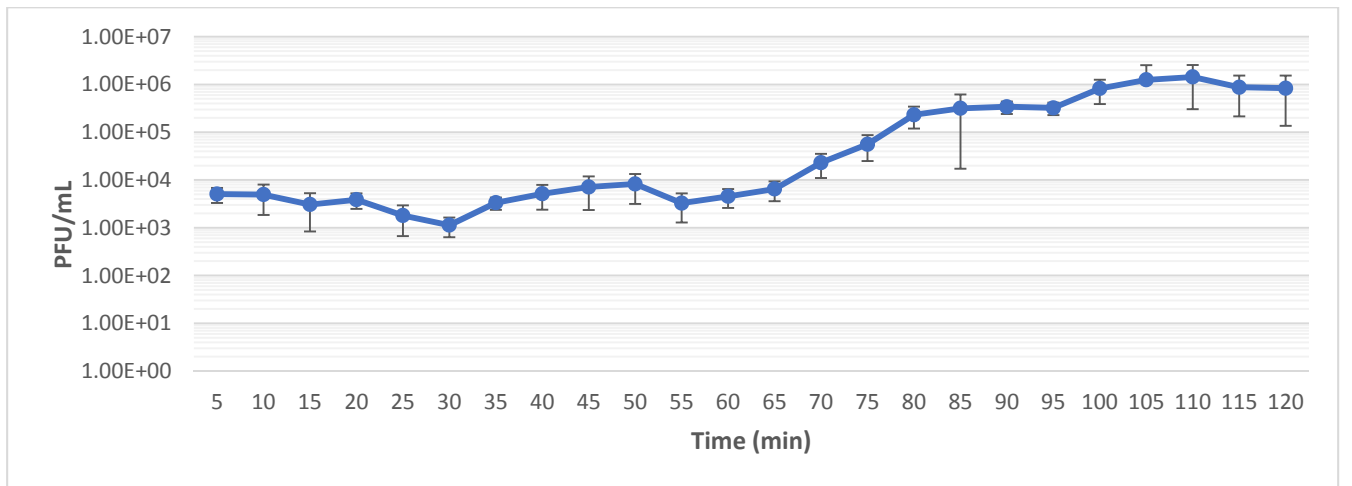
| Isolate | Isolation source | Cavity formation on CVP medium (25 °C, 48 hrs) | Growth on NA at 37°C, 48 hrs | Production of reducing substance from sucrose | Acid production of reducing substance from sucrose | PCR - <i>Pel</i> gene (Darasse et al 1994) | PCR - <i>P. atrosepticum</i> (De Boer & Ward 1995) | PCR - <i>P. carotovorum</i> subsp. <i>carotovorum</i> (Kang et al 2003) | MALDI-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)

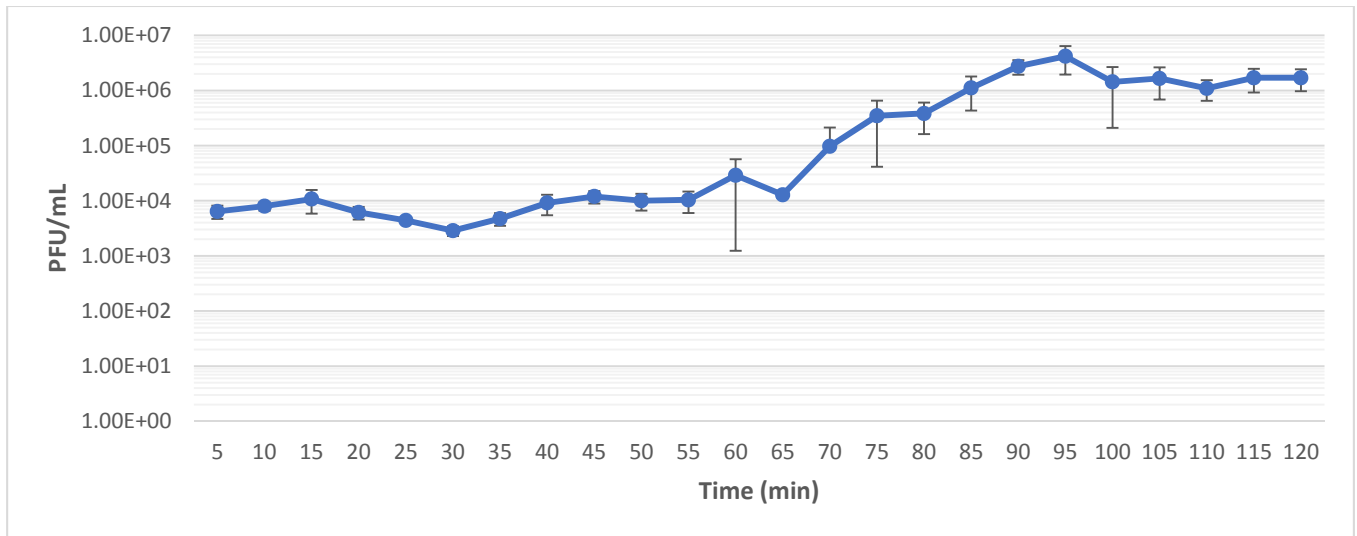


Figure S1. Single step growth curve growth analysis of phage CB1 infection of *P. atrosepticum* strain DSM18077 (A), phage CB3 infection of *P. atrosepticum* strain DSM30186 (B) and phage CB4 infection of *P. atrosepticum* DSM30186 (C). Each assay was independently repeated in triplicate and the results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

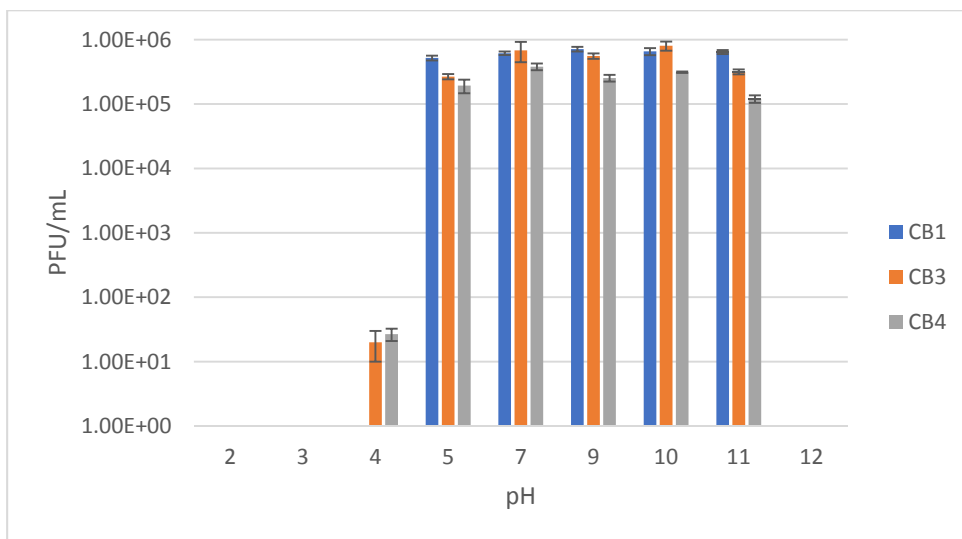


Figure S2. Stability of *Pectobacterium* phages CB1, CB3 and CB4 to various pH values upon 24 hours of exposure. Error bars represent standard deviation of biological repeats (n=3).

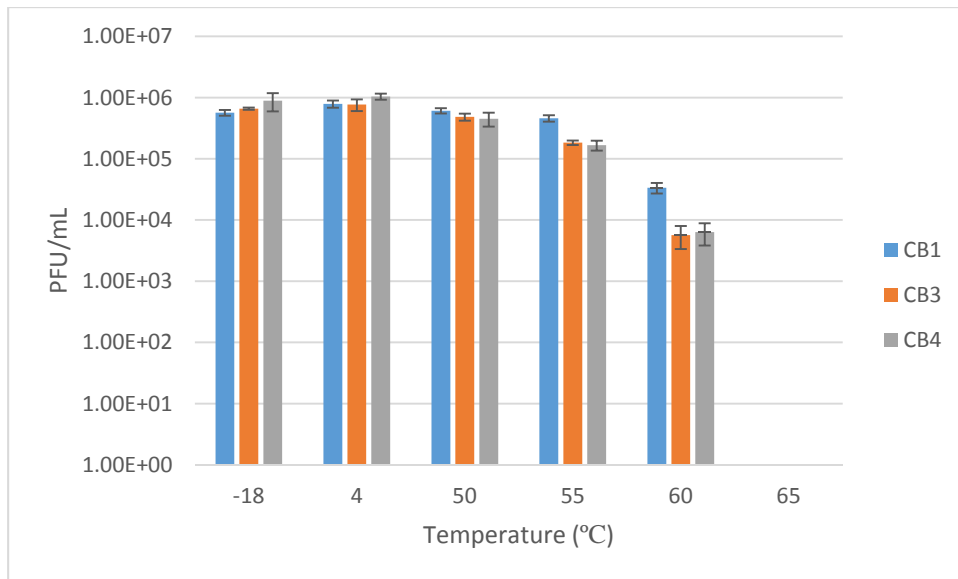


Figure S3. Stability of *Pectobacterium* phages CB1, CB3 and CB4 to various temperatures upon one-hour exposures. Error bars represent standard deviation of biological repeats (n=3).

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 description |
|-------------------------------|-------------------------------|--------------------------------|--|
| 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 | TCGGACCG <u>CACGTATCGTGC</u> AGCCCCTTA |
| 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 | TCGGACCG <u>CACGTATCGTGC</u> AGCCCCTTA |

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 | GCCCCTTCGGTGGGCTTTTTTATCTAT | -14.7 |
| Torf1_76 | 46,958 - 46,987 | GCCCCTCGATTGAGGGGCTTATTTTTTAG | -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 | CTCCCTCTTCAGAGGGAGcTTTTAAACCTGA | -12.1 |
| Torf1_88 | 70,620 - 70,649 | GCCCCACTTCGGTGGGGCTTTTTCCGTTAT | -18 |
| Torf1_89 | 71,047 - 71,076 | GCCCCGATTAAGGGGGCTTTTTTATTACA | -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 | TAACCCCTTCGGGGTATCTTATTTTTTA | -13.6 |
| Torf3_21 | 7,283 - 7,314 | GCCATCCCTTCGGGGATGGCTGTTTTATTGAG | -19.3 |
| Torf3_29 | 11,386 - 11,422 | CGGACTCCCATCTTAGGGAGTCCGTATTTTTTCAT A | -17.1 |
| Torf3_57 | 20,329 - 20,357 | GCCAGATTTAATCTGGCTTTTTCTTTTTA | -9.4 |
| Torf3_49 | 25,440 - 25,472 | GCCAGCCCTTCGGGGCTGGTTTTTTAATATCAT | -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 | GCCACCTAGTGTGGGCTTATATTAATCT | -10.7 |
| Torf3_65 | 33,840 - 33,870, complement | GGGAGCCTAATGGCTCCCTTTTTAATCTGGA | -15.4 |
| Torf3_77 | 45,360 - 45,387 | GCCCACTTCGGTGGGCTTTTTTATCTAT | -14.7 |
| Torf3_81 | 47,267 - 47,296 | GCCCCTCGATTGAGGGGCTTATTTTTTAG | -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 | GCCGGGATAATTCCCCGGCTTTTTTATATCTA | -18.4 |
| Torf3_92 | 69,948 - 69,978 | CTCCCTCTCAGAGGGAGcTTTTAAACCTGA | -12.1 |
| Torf3_94 | 70,802 - 70,831 | GCCCCACTTCGGTGGGGCTTTTTCCGTTAT | -18 |
| Torf3_95 | 71,229 - 71,258 | GCCCCGATTAAGGGGGCTTTTTTATTACA | -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 | GCCAGATTTAATCTGGCTTTTCTTTTTAA | -9.4 |
| Torf4_48 | 25,226 - 25,258 | GCCAGCCCTTCGGGGCTGGTTTTTTAATATCAT | -18.8 |
| Torf4_50 | 28,183 - 28,208 | GCCCCTTCGGGGGCTTTTTTGAGGCT | -13.7 |
| Torf4_54 | 29,592 - 29,620 | AGCCCCTAACGGGGCTTTTTATTGAGGT | -12.8 |
| Torf4_59 | 31,860 - 31,888 | GCCCACCTAGTGTGGGCTTATATTAATCT | -10.7 |
| Torf4_64 | 33,826 - 33,656, complement | GGGAGCCTAATGGCTCCCTTTTTAATCTGGA | -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 | GCCCCTCGATTGAGGGGCTTTATTTTTAG | -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 | GCCCCGATTAAGGGGCTTTTTTATTACA | -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 |

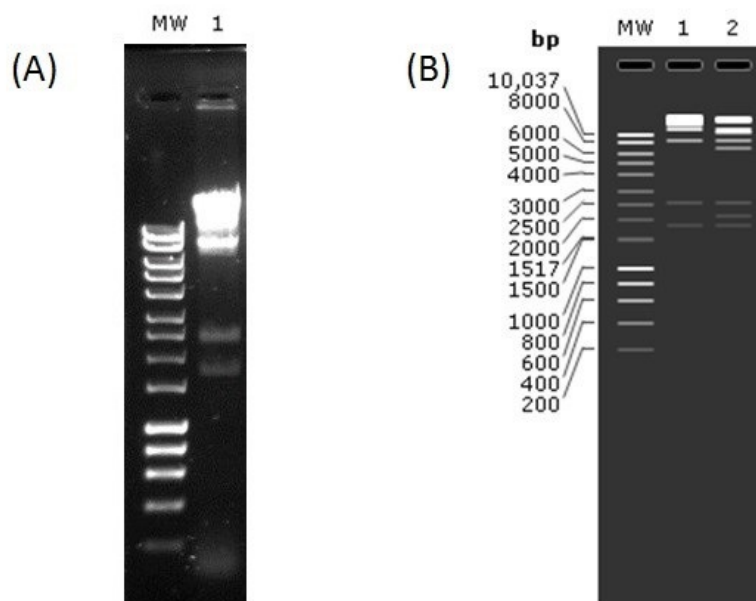


Figure S4. (A) Genomic DNA of *Pectobacterium* phage CB1, which had been digested with restriction enzyme *Clal* (lane 1), with DNA marker (Hyperladder 1kb, Bioline) (lane MW). (B) *In silico* digest of CB1 redundant genomic DNA with *Clal* with Dam methylation (lane 2); non-Dam methylation (lane 3);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

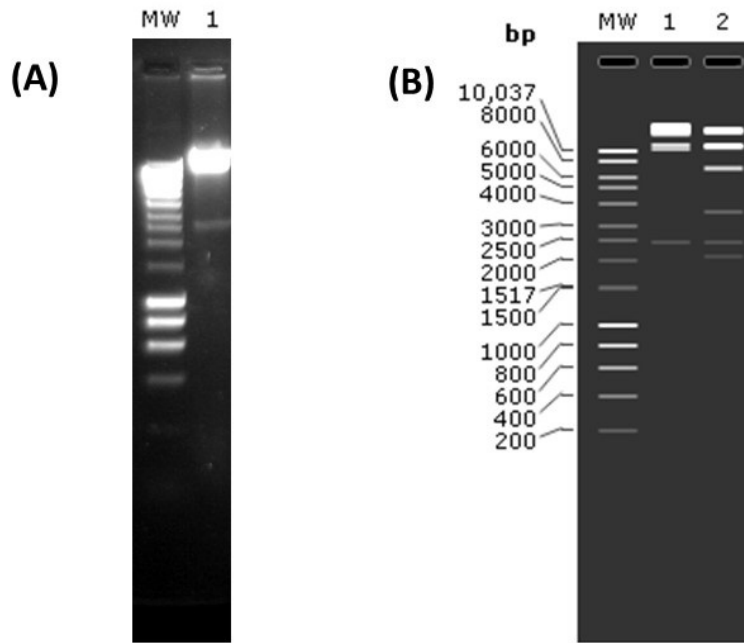


Figure S5. (A) Genomic DNA of *Pectobacterium* phage CB3, which had been digested with restriction enzyme *Cl*I (lane 1), with DNA marker (Hyperladder 1kb, Bioline) (lane MW). (B) *In silico* digest of CB3 redundant genomic DNA with *Cl*I with Dam methylation (lane 2); non-Dam methylation (lane 3); DNA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

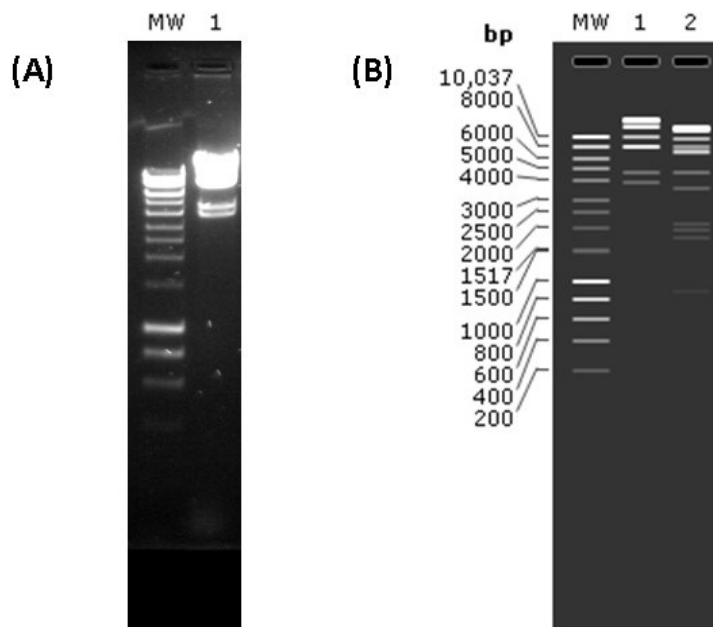


Figure S6. (A) Genomic DNA of *Pectobacterium* phage CB4, which had been digested with restriction enzyme *Cl*I (lane 1), with DNA marker (Hyperladder 1kb, Bioline) (lane MW). (B) *In silico* digest of CB4 redundant genomic DNA with *Cl*I with Dam methylation (lane 2); non-Dam methylation (lane 3); DNA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

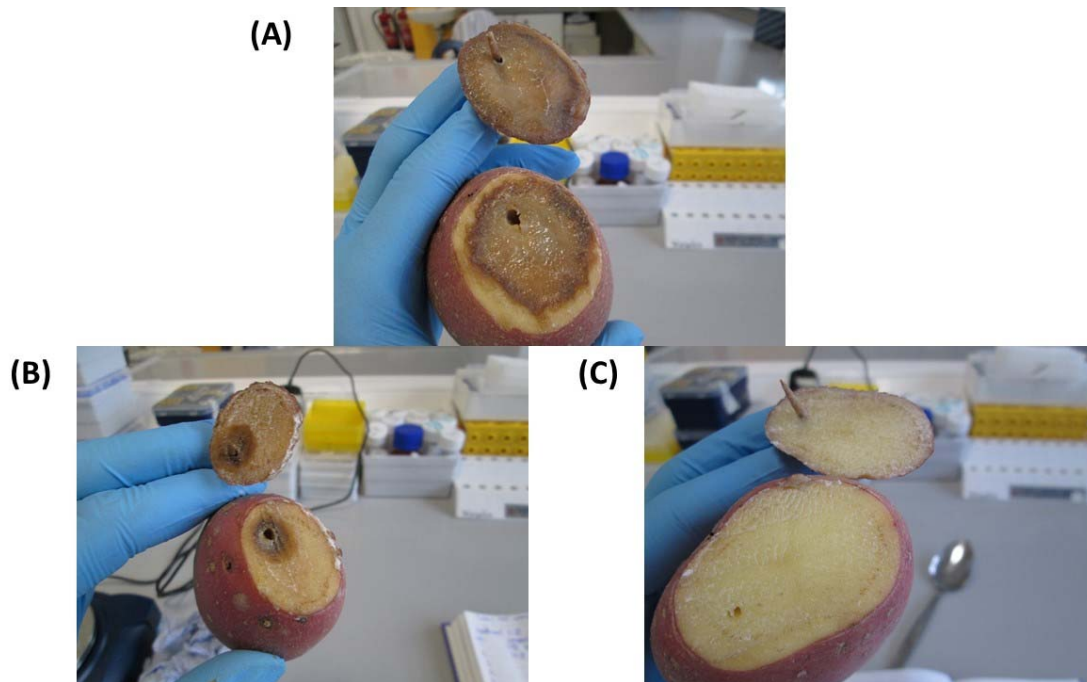


Figure S7. Pictures of the typical observed outcomes for the tuber rot assays. A: tuber treated with bacterial strains DSM 18077 + DSM 30186 and SM buffer. B: tuber treated with bacterial strains DSM 18077 + DSM 30186 and phage mixture (CB1 + CB3 + CB4). C: tuber treated with water and SM buffer.