

Supplementary Figures

Molecular, physiological and phylogenetic traits of *Lactococcus* 936-type phages from distinct dairy environments

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Supplementary Figure S1. Multiple sequence alignment of putative DNA polymerase subunits of *Lactococcus* phages sequenced in this study and phages bIBB29, R3.4 and P680 as the nearest homologues. Similar residues are coloured according to their conservation based on BLOSUM62 scoring (max: 3.0, light blue; low: 0.5, grey).

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Eg1      MTS3SLFDK3VSTAKELKES3dDFAG3G3LL3---W3NV3kDIL3PKG3SLGLITGSEK3SMK3SSLAQDLA3Q
bIBB29  MTS3SLFDK3VSTAKELKES3dDFAG3G3LL3---W3NV3kDIL3PKG3SLGLITGSEK3SMK3SSLAQDLA3Q
5g1     MTS3SLFDK3VSTAKELKES3EDFSG3G3LL3---W3NV3QDIL3PKG3SLGLITGSEK3SMK3SSLAQDLA3Q
24tp1  MTS3SLFDK3VSTAKELKES3EDFSG3G3LL3---W3NV3QDIL3PKG3SLGLITGSEK3SMK3SSLAQDLA3Q
77s    MTS3SLFDK3VSTAKELKES3EDFSG3G3LL3---W3NV3QDIL3PKG3SLGLITGSEK3SMK3SSLAQDLA3Q
14s    MTS3SLFDK3VSTAKELKES3EDFSG3G3LL3---W3NV3QDIL3PKG3SLGLITGSEK3SMK3SSLAQDLA3Q
F14    MT3n3iF3nKV3qTAK3hLKE3rED3lin--LKDD3W3lid3tlmP3ssq3aGilv3apfK3SfK3SSLAmhm3Al
P680   MT3n3iF3eKV3qTAK3hLKE3rED3lin--LKDD3W3lid3tlmP3ssq3aGilv3apfK3SfK3SSLAmhm3Al
F12    MT3n3iF3nKV3qTAK3hLKE3rED3lin--LKDD3W3lid3tlmP3ssq3aGilv3apfK3SfK3SSLAmhm3Al
F13    MT3n3iF3nKV3qTAK3hLKE3rED3lin--LKDD3W3lid3tlmP3ssq3aGilv3apfK3SfK3SSLAmhm3Al
R3.4   MT3n3iF3DKV3qTAK3hLKE3rED3lin--LKDD3W3lid3tlmP3ssq3aGilv3apfK3SfK3SSLAmhm3Al

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5g1     AMALGEPFAGRETTKTNVLFIQNENSRLTEHQRLKGSRRDSPDNLYFLHGGAFKLD3TWKY3
24tp1  AMALGEPFAGRETTKTNVLFIQNENSRLTEHQRLKGSRRDSPDNLYFLHGGAFKLD3TWKY3
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F14    AMALGEPFAGRETTKTNVLFIQNENSRLTEHQRLKGSRRDSPDNLYFLHGGAFKLD3TWKY3
P680   mv3sq3G3lP3ffG3ydT3kr3sktLy3Id3NE3dt3drelnkR3Lr-nk3dta3Ped3Lh3FL3tGG3eF3mLD3----
F12    mv3sq3G3lP3ffG3ydT3kr3sktLy3Id3NE3dt3drelnkR3Lr-nk3dta3Ped3Lh3FL3tGG3eF3mLD3----
F13    mv3sq3G3lP3ffG3ydT3kr3sktLy3Id3NE3dt3drelnkR3Lr-nk3dta3Ped3Lh3FL3tGG3eF3mLD3----
R3.4   mv3sq3G3lP3ffG3ydT3kr3sktLy3Id3NE3dt3drelnkR3Lr-nk3dta3Ped3Lh3FL3tGG3eF3mLD3----

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F13    DS3----Hh3mn3L3--LY3ey3Ik3dn3DI3kf3VIL3Dn3Lmt3mL3rng3DII3yg3kd-fep3mL3Rr3IT3rL3kl3l
R3.4   DS3----Hh3mn3L3--LY3ey3Ik3En3DI3kf3VIL3Dn3Lmt3mL3rng3DII3yg3kd-fep3mL3Rr3IT3rL3kl3l

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Eg1      LDM3KHD3KY3VT3FMIVAHARKQAGEQSLTERDFRIIPSHILGATTIP3SWYE3iAFT3MSPK3INS3
bIBB29  LDM3KHD3KY3VT3FMIVAHARKQAGEQSLTERDFRIIPSHILGATTIP3SWYE3iAFT3MSPK3INS3
5g1     LDM3KHD3KY3VT3FMIVAHARKQsGEQSLTERDFRIIPSHILGATTIP3SWYE3iAFT3MSPK3INS3
24tp1  LDM3KHD3KY3VT3FMIVAHARKQAGEQSLTERDFRIIPSHILGATTIPAWYE3vAFT3MSPK3INS3
77s    LDM3KHD3KY3VT3FMIVAHARKQAGEQSLTERDFRIIPSHILGATTIPAWYE3vAFT3MSPK3INS3
14s    LDM3KHD3KY3VT3FMIVAHARKQAGEQSLTERDFRIIPSHILGATTIPAWYE3vAFT3MSPK3INS3
F14    LDM3KHD3KY3VT3FMIVAHARKQAGEQSLTERDFRIIPSHILGATTIPAWYE3vAFT3MSPK3INS3
P680   f3-----qd3VTF3l3lVAH3An3Ks3Ayan3S3mddkaymvk3PS3da3LG3gs3Tlt3AW3eF3m3lml3SPK3--r
F12    f3-----qd3VTF3l3lVAH3An3Ks3Ayan3S3mddkaymvk3PS3da3LG3gs3Tlt3AW3eF3m3lml3SPK3--r
F13    f3-----qd3VTF3l3lVAH3An3Ks3Ayan3S3mddkaymvk3PS3da3LG3gs3Tlt3AW3eF3m3lml3SPK3--r

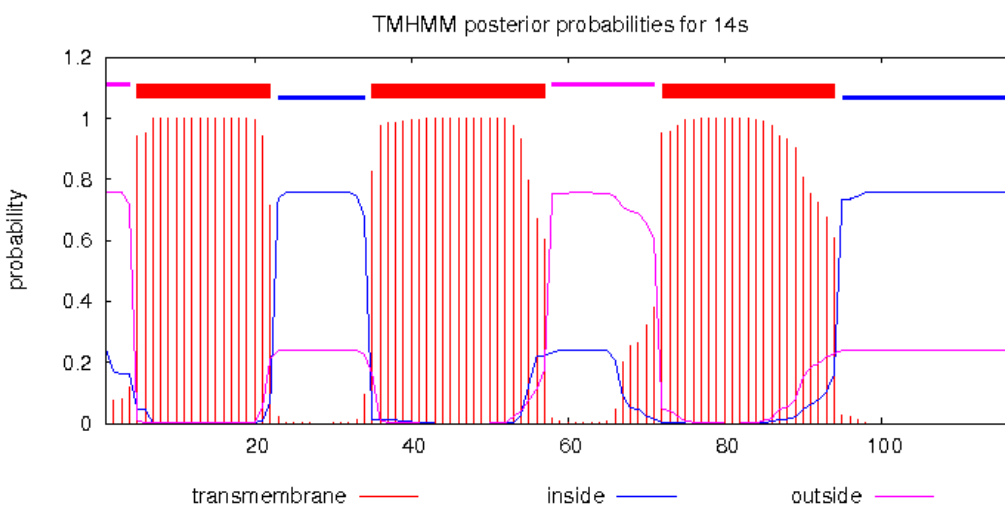
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5g1 KTKNrYSIMKVFARNFAFNnEILWGYVGSFAFTSIEQDKKEPDSELVEKVKRETPIETTKE
24tp1 KTKNgYSIMKVFARNFAFNnEILWGYVGSvFTSIEQDKKEPDSELiEeVKqETPIETTKE
77s KTKNgYSIMKVFARNFAFNnEILWGYVGSFAFTSIEQDKKEPDSELVEKVKRETPIETTKE
14s KTKNgYSIMKVFARNFAFNnEILWGYVGSFAFTSIEQDKKEPDSELVEKVKRETPIETTKE
F14 KTKNgYSIMKVFARNFAFNnEILWGYVGSFAFTSIEQDKKEPDSELVEKVKRETPIETTKE
P680 gkhNdfSklsVkarGyqFdDdlfnsYVdSvFTcvnksKKEPDSELiEeVKqETPIETTKE
F12 gkyNdfSklsVkarGyqFdDdlfnsYVdSvFTcvnksKKEPDSELVEKVKRETPIETTKE
F13 gkyNdfSklsVkarGyqFdDdlfnsYVdSvFTcvnksKKEPDSELVEKVKRETPIETTKE
R3.4 gkhNdfSklsVkarGyqFdDdlfnsYVdSvFTcvnksKKEPDnELVEKVKaETPIETTKE

Eg1 SAQAFDLAKEQGKVIEND
bIBB29 SAQAFDLAKEQGKVIEND
5g1 SAQAFDLAKEQGKVIEND
24tp1 SAQAFDLAKEQGKVIEND
77s SAQAFDLAKEQGKVIEND
14s SAQAFDLAKEQGKVIEND
F14 SAQAFDLAKEQGKVIEND
P680 SAQAFDLAKEQGKVtEND
F12 SAQAFDLAKEQGKVIEND
F13 SAQAFDLAKEQGKVIEND
R3.4 SAQAFDLAKEQGKVIEND

Supplementary Figure S2. Identification of transmembrane domains of the putative holins of *Lactococcus* phages sequenced in this study. Prediction of transmembrane domains was performed using the TMHMM tool (Krogh et al., 2001).



Supplementary Figure S3. Multiple sequence alignment of receptor binding proteins (RBPs) of

Lactococcus phages sequenced in this study with protein representative of 936-type RBP

subgroups recognized by Murphy et al. (2016). Similar residues are coloured according to their conservation based on BLOSUM62 scoring (max: 3.0, light blue; min: 0.5, grey).

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645 MTIKkFTFFSPNGTEFPVsANaDAKLYMLLSEKDYSQFliRHWETPINTALNRIYTNTSL
340 MTIKkFTFFSPNGTEFPVsANaDAKLYMLLSEKDYSQFliRHWETPINTALNRIYTNTSL
P475 MTIKkFTFFSPNGTEFPVsANaDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
77s MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTdLNRIYkNTSL
bIBB29 MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
24tp1 MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
5g1 MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
F12 MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
F13 MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
F14 MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
Eg1 MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
14s MTIKNFTFFSPNGTEFPVGANNDAKLYMLLSEKDYSQFslRHWETPINTALNRIYkNTSL
936 MTIKNFTFFSPNGTEFPVGSNNDgKLYMmLtgmDYktiRRkdWlTptNsgLNvIYnNTSi
sk1 MTIKNFTFFSPNsTEFPVGSNNDgKLYMmLtgmDYrtiRRkdWssPlNTALNvqYTNTSi
p2 MTIKNFTFFSPNsTEFPVGSNNDgKLYMmLtgmDYrtiRRkdWssPlNTALNvqYTNTSi
712 MTIKNFTFFSPNGTEFPVGSNNDAKLYMmLtgmDYktiRRkdWvePlNTsLNvMYpNTSi
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P008 MTIKNFTFFSPNGTEFPVGSNNDgKLYMmLtgmDYgtiRRkdWtsPlNTALNvqYTNTSi
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bIL170 MTIKNFTFFSPNGTEFPVGSNNDgKLYMmLagmDYgtiRRkdWtsPlNTALNvqYTNTSi
PhiL.6 MTIKNFTFFSPNGTEFPVGSNNDgKLYMmLtgmDYgtiRRkdWtsPlNTALNvqYTNTSi
Phi155 MTIKyFTFFSPNnTEFPVGSNNDAKLYMmLtgmDYgtiRRkdWsaPVNTALNvqYTNTSi
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645 LVGGRYFELYNEAVALNSNATNFVHANIDISNvTNPVTiSVETSsnNSNkVDINTGVGVLK
340 LVGGRYFELYNEAVALNSNATNFVHANIDISNvTNPVTiSVETSsnNSNkVDINTGVGVLK
P475 LVGGRYFELYdEAVALNSNATNFVHANIDISNaTNPVTmSVETSdNSNQVDINTGVGVLK
77s LVGGRYFELYNEAVALNSNATNFVHANIDISNaTNPVTmSVETSdNSNQVDINTGVGVLK
bIBB29 LVGGRYFELYNEAVALNSNATNFVHANIDISNaTNPVTmSVETSdNSNQVDINTGVGVLK
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p2 iaGGRYFELlNetVALkgdsvNyiHANIDltqTanPVsLSaETanNSNgVDInNsGVLK
712 iaGGRYFELlsEtVALkaNsvNyiHANIDltkTanPVsLSaEnvDNSNnVdfNnrsGVLK
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PhiL.6 iaGGRYFELsNetVALkgNsvNyiHANIDltqTahPVsLSaETinNSNhVDlNnssGVLK
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Phi155 iaGGRYFELvgEtipLkaNsvNyiHANIDltqTTsPVkLSaETlnNSNrtDINnssGVLK

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340 KCIEVVETNAiGVSAVTKPSGSSILEKIYP---VGAYYfSSQPTEPATLF-----GFGT
P475 KCIEVVETNAMGVSAVTKPSGSSiiEKIYP---VGAYYLSSQPTEPATLF-----GFGT
77s KCIEVVETNAMGVSAVTKPSGSSiiEKIYP---VGAYYLSSQPTEPATLF-----GFGT
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14s KCIEVVETNAMGVSAVTKPSGSSiiEKIYP---VGAYYLSSQPTEPATLF-----GFGT
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bIL170 vvIdirtTNgtGVisaeQptehtLLddVi-----Ins--LvnkkdiPwTdl-NrASGvGs
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Phi155 vlIdirtTNGLGVisseiPkqittLdelatKTANledltvkgdikgwtsVs----mqnva

645 WSRVKGRGLvGVD-ESDSALsSGGKQG-----GS-----TNPLSQHTITPSSGQFVVARG
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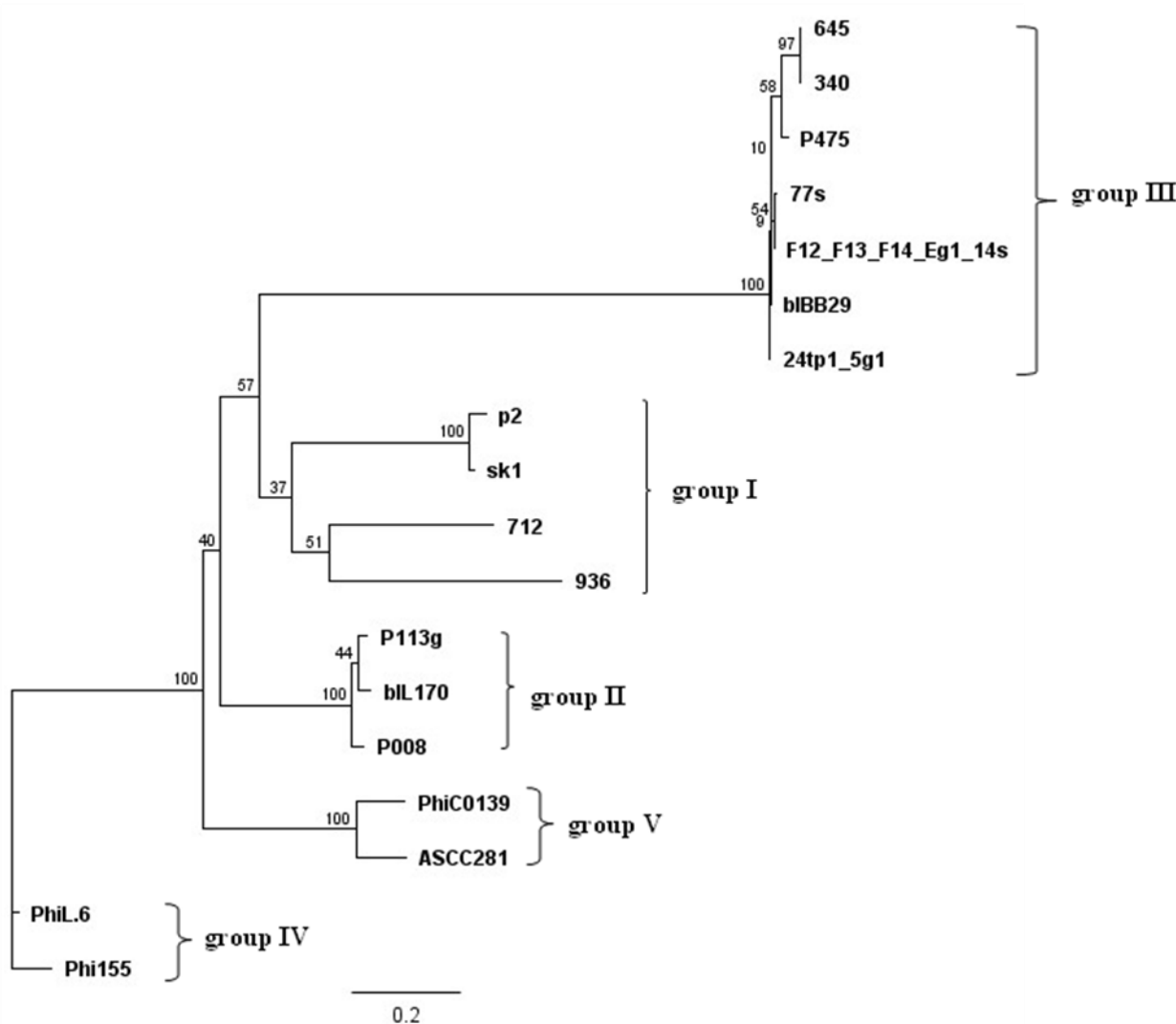
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5g1 AGKWHWSSGGAPSNSYAMDTENGGV--TVGDNTNHNNWQPFEAAYIWKRIN-----

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14s      AGKWHWSSGGAPSNSYAMDTENGGI--TVGDNTNHNNWQPFEAAYIWKRIN-----
936      tliYnhdltipskfSwllhtNGLI-----DlfsngN--ikttdYIltsgqFwiTkdkLpe
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p2       ---gHfAg---rdtSFhiDinpnGsitwwGaNidktp--iatrgngsyfIk-----
712      ---gHfpt---tSNSFhiDlEhdGtfrwwGEDkas---gsvrgtamyfIk-----
PhiC0139 t--gy--tkGhdSqaiAMpTsaGftglkkGqqytfNk--rdvsgraggatrvkiiVkJyrN
ASCC281  ---gytNghnSqalampakaiysGL--kkGqqytfdk--rdasglaggskhemmiVkJyrN
P008     ta-gtfyShGnlSlSl-inmspsGI--aVGn-----PnntsmngKtIsFvLSVPLL--
P113g    ta-gtfySpGhlSlSl-inmspsGI--aVGn-----PnntsmngKtIsFaLSaPLL--
bIL170   ss-gtfyShGnlSlSl-inmspsGI--aVGn-----PnntsmngKtIsFaLSaPLL--
PhiL.6   emttqrAnnnnkpmelliDTngtis--vwsysags---gnyggvVgtyfq-----
Phi155   emttqrAnnnnkpmelqiDTngtis--vwsysags---gnyggvVgtyfq-----

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Supplementary Figure S4. Maximum-likelihood phylogenetic tree of receptor binding proteins (RBPs) of *Lactococcus* phages sequenced in this study. RBP phylogenies were reconstructed from multiple alignment of complete protein sequences, which were generated using MUSCLE with 8 iterations (default settings). A maximum-likelihood tree was constructed using PHYML and the Junker-Castor substitution model with 100 bootstrap resamplings. The scale bar represents evolutionary distance. Consensus support (%) is marked at each node.



Supplementary Figure S5. Multiple sequence alignment of putative TpeX proteins of *Lactococcus* phages sequenced in this study with the TpeX identified in other 936-type phages (Murphy et al., 2016). Similar residues are coloured according to their conservation based on BLOSUM62 scoring (max: 3.0, light blue; min: 0.5, grey).

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Phi145      -----mLNf-----SsgvtfGkhygwpagtatpsKnraysqkvkvtvpndqytisA
Phi93       -----mLNf-----SsgvtfGkhygwpagtatpsKnraysqkvkvtvpndqytisA
Eg1         MTVYPNLNLLKNRTRTTSATSTSSAWGTLFSSEQIYDSAIAKSKTGVSAMTLGF DVSVPLNA
24tp1      MTVYPNLNLLKNRTRTTSATSTSSAWGTLFSSEQIYDSAIAKSKTGVSAMTLGF DVSVPLNA
F14        MTVYPNLNLLKNRTRTTSATSTSSAWGTLFSSEQIYDSAIAKSKTGVSAMTLGF DVSVPLNA
5g1        MTVYPNLNLLKNRTRTTSATSTSSAWGTLFSSEQIYDSAIAKSKTGVSAMTLGF DVSVPLNA
F12        MTVYPNLNLLKNRTRTTSATSTSSAWGTLFSSEQIYDSAIAKSKTGVSAMTLGF DVSVPLNA
F13        MTVYPNLNLLKNRTRTTSATSTSSAWGTLFSSEQIYDSAIAKSKTGVSAMTLGF DVSVPLNA
77s        -----NLLKNRTRTTSATSTSSAWGTLFSSEQIYDSAIAKSKTGVSAMTLGF DVSVPLNA
14s        MTVYPNLNLLKNRTRTTSATSTSSAWGTLFSSEQIYDSAIAKSKTGVSAMTLGF DVSVPLNA

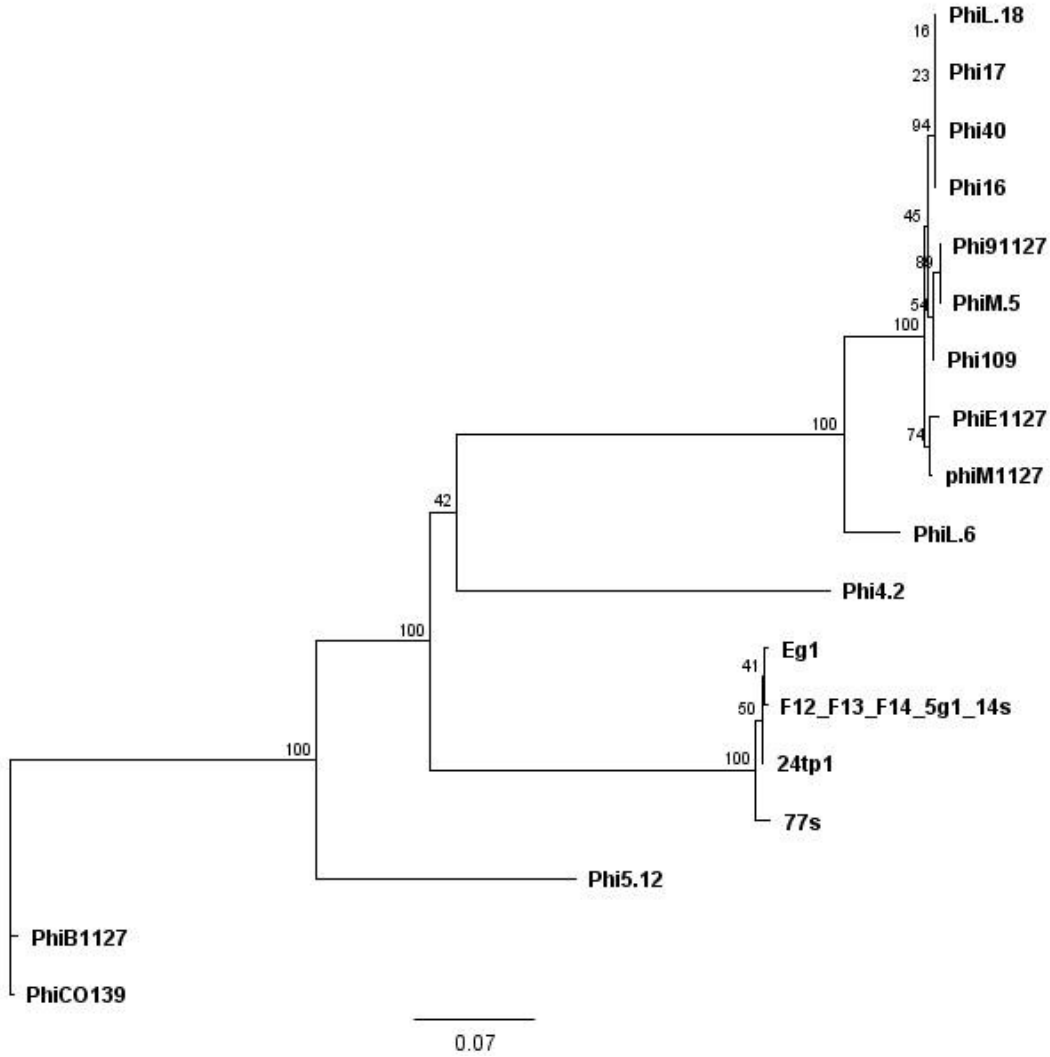
Phi145      dwkNanwlsiyGftnegdttavAr-ygsanGtGTGSwdsnfvggytaandmtftVpdnvn
Phi93       dwkNanwlsiyGftnegdttavAr-ygsanGtGTGSwdsnfvggytaandmtftVpdnvn
Eg1         KVGNNISVQLKGQNSQAHGNV GANDFNTIVGE---KWHNIEQSDLGKkIRLSTSVELDPK
24tp1      KVGNNISVQLKGQNSQAHGNV GANDFNTIVGE---KWHNIEQSDLGKkIRLSTSVELDPK
F14        KVGNNISVQLKGQNSQAHGNV GANDFNTIVGE---KWHNIEQSDLGKkIRLSTSVELDPK
5g1        KVGNNISVQLKGQNSQAHGNV GANDFNTIVGE---KWHNIEQSDLGKkIRLSTSVELDPK
F12        KVGNNISVQLKGQNSQAHGNV GANDFNTIVGE---KWHNIEQSDLGKkIRLSTSVELDPK
F13        KVGNNISVQLKGQNSQAHGNV GANDFNTIVGE---KWHNIEQSDLGKkIRLSTSVELDPK
77s        KVGNNISVQLKGQNSQAHGNV GANDFNTIVGE---KWHNIEQSDLGKkIRLSTSVELDPK
14s        KVGNNISVQLKGQNSQAHGNV GANDFNTIVGE---KWHNIEQSDLGKkIRLSTSVELDPK

Phi145      YivasyAskspDtlTlq-----GllngKpKLEqGSkvTPWmrSSSEtiesDYPSYIGnY
Phi93       YivasyAskspDtlTlq-----GllngKpKLEqGSkvTPWmrSSSEtiesDYPSYIGnY
Eg1         YqSFDALADTDSITIRQVEGTPGLVYSKLEPGSTDPWIPSSSEVTAEDYPSYIGTY
24tp1      YqSFDALADTDSITIRQVEGTPGLVYSKLEPGSTDPWIPSSSEVTAEDYPSYIGTY
F14        YHSFDALADTDSITIRQVEGTPGLVYSKLEPGSTDPWIPSSSEVTAEDYPSYIGTY
5g1        YHSFDALADTDSITIRQVEGTPGLVYSKLEPGSTDPWIPSSSEVTAEDYPSYIGTY
F12        YHSFDALADTDSITIRQVEGTPGLVYSKLEPGSTDPWIPSSSEVTAEDYPSYIGTY
F13        YHSFDALADTDSITIRQVEGTPGLVYSKLEPGSTDPWIPSSSEVTAEDYPSYIGTY
77s        YHSFDALADTDSITIRQVEGTPGLVYSKLEPGSTDPWIPSSSEVTAEDYPSYIGTY
14s        YHSFDALADTDSITIRQVEGTPGLVYSKLEPGSTDPWIPSSSEVTAEDYPSYIGTY

Phi145      TgKivdgQSTDPvrYnWKKIE
Phi93       TgKivdgQSTDPvrYnWKKIE
Eg1         TDKNSNEQSTDPEKYTWKKIE
24tp1      TDKNSNEQSTDPEKYTWKKIE
F14        TDKNSNEQSTDPEKYTWKKIE
5g1        TDKNSNEQSTDPEKYTWKKIE
F12        TDKNSNEQSTDPEKYTWKKIE
F13        TDKNSNEQSTDPEKYTWKKIE
77s        TDKNSNEQSTDPEKYTWKKIE
14s        TDKNSNEQSTDPEKYTWKKIE

```


Supplementary Figure S6. Maximum-likelihood phylogenetic tree of MTP extension of *Lactococcus* phages sequenced in this study with the MTP extensions identified in other 936-type phages (Murphy et al., 2016). MTP extension phylogenies were reconstructed from multiple alignment of major tail protein extension sequences generated using MUSCLE with 8 iterations (default settings). A maximum-likelihood tree was built using PHYML and the Junker-Castor substitution model with 100 bootstrap resamplings. The scale bar represents evolutionary distance. Consensus support (%) is marked at each node.



References

Krogh, A., Larsson, B., von Heijne, G. & Sonnhammer, E. L. L. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *J. Mol. Biol.* **305**, 567-580 (2001).

Murphy, J. et al. Comparative genomics and functional analysis of the 936 group of lactococcal *Siphoviridae* phages. *Sci. Rep.* **6**, 21345 (2016).

Supplementary Tables.

Supplementary Table S1. Host range studies of *Lactococcus* phages sequenced in this study tested on industrial, laboratory and environmental *L. lactis* strains. Grey-shaded boxes indicate clear plaques; light grey boxes indicate turbid plaques formed on the specified strains.

	Bacteriophages							
	Eg1	F12	F13	F14	5g1	14s	77s	24tp1
Industrial strains								
<i>L. lactis</i> subsp. <i>cremoris</i>								
IBB694								
IBB695								
IBB696								
IBB697								
IBB698								
IBB699								
IBB700								
IBB1788								
IBB1789								
IBB1790								
IBB1791								
IBB1792								
<i>L. lactis</i> subsp. <i>lactis</i>								
IBB743								
IBB749*								
IBB750*								
IBB752								
IBB753								
IBB754								
IBB757								
IBB758								
IBB759								
IBB761								
IBB763								
IBB764								
IBB1784								
IBB1796								
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i>								

IBB734*
IBB735*
IBB736*
IBB737*
IBB738*
IBB742
IBB744
IBB745
IBB746
IBB747
IBB748*
IBB751*
IBB755
IBB756
IBB760
IBB762
IBB1286*

Natural isolates

L. lactis subsp. *cremoris*

IBB338
IBB339
IBB340
IBB341
IBB342
IBB704

L. lactis subsp. *lactis*

10LC

other *Lactococcus* species

L. garvieae 3LC
L. plantarum 76B
L. laudensis 115
L. raffinolactis 87B

Laboratory strains

L. lactis subsp. *cremoris*

LM0230
MG1363
IBB477
NZ9000

L. lactis subsp. *lactis*
biovar. *diacetylactis*

IL1403

F7/2

*The industrial starter strains used at the time of whey sample collection.