

Motif ExoI

Enterococcus phage vB_Efae230P-4 1 MNAE EHAKWLDGTFEDEFERYK---QKIQIQDCCDIEETFYNNWQQAQPKMKSRMFTCASWHNEKVTI---TVAFDFRYFFDAHYHAKCNK
Enterococcus phage vB_IME195 1 MNAE EHAKWLDGTFEDEFERYK---QKIQIQDCCDIEETFYNNWQQAQPKMKSRMFTCASWHNEKVTI---TVAFDFRYFFDAHYHAKCNK
Streptococcus phage C1 1 -----MKIEFQYKSPFGQKVTSDVLYCDIEETAFINKNNGSQKHASTYHSEFTSLAVSFKTEGEPVVFNFHKQKLF-----DIEK--S
Staphylococcus phage P68 1 -----MGL-----HKHEEMILYWDITLAKNKNNGKKEETVYNNVTYVAIGQNGYI---DVEVFPSEETDAVYTVKLRDT
Streptococcus phage Cp-1 1 -----MTCYAGFETITNEEET-----EVLVLFKAVIDYKLDLDFKNLSLDEKLSLYLDL---DK
Bacillus phage phi 29 1 -----MPRMMSCHEETFKVEDC-----RVAHGYMNI---DHSEYKGNLSLDEPMAMV-----
Actinomyces phage Av1 1 ---WR-----QFIAS---PARGVRRSHKIPSCAHEETDEDDC-----RVWSGIQIV---GKLQNYDGLSLDGMMSHI-----

Motif ExoII

Enterococcus phage vB_Efae230P-4 94 RKGE PNNRRLIMYHNGNKFDNHFIAKMHVDVNA-EYNNQDEK---SEITOKYASHMSIKKEENYILB-KRVKGISHSE-SEKVRDIEIEEDTV
Enterococcus phage vB_IME195 94 RKGE PNNRRLIMYHNGNKFDNHFIAKMHVDVNA-DYNNQDEK---SEITOKYASHMSIKKEENYILB-KRVKGISHSE-SEKVRDIEIEEDTV
Streptococcus phage C1 80 KTI--RKSIEFRLHNGAKFDNHFIAKMHVDVNA-DYNNQDEK---SEITOKYASHMSIKKEENYILB-KRVKGISHSE-SEKVRDIEIEEDTV
Staphylococcus phage P68 94 KTI--RKSIEFRLHNGAKFDNHFIAKMHVDVNA-DYNNQDEK---SEITOKYASHMSIKKEENYILB-KRVKGISHSE-SEKVRDIEIEEDTV
Streptococcus phage Cp-1 75 IT--KSKTDDIMLHNGNKFDNHFIAKMHVDVNA-DYNNQDEK---SEITOKYASHMSIKKEENYILB-KRVKGISHSE-SEKVRDIEIEEDTV
Bacillus phage phi 29 57 TYTETGDEFFIYFHH-LKFGGSEFTFLNLDI-----PCTYETNDMGWYSLTEGEP---FTTFFRPSI
Actinomyces phage Av1 49 -----LKVQADLYFHH-LKFGGSEFTFLNLDI-----PCTYETNDMGWYSLTEGEP---FTTFFRPSI
68 -----SEYASHELYFHH-LKFGGSEFTFLNLDI-----PCTYETNDMGWYSLTEGEP---FTTFFRPSI

Motif ExoIII

Enterococcus phage vB_Efae230P-4 187 MKTGCSIAACGIMLEAGGLKPKDOLKTTFDYKHYHQENMDEBAQKVAVDLIVQL EEEETFYQNDITLSSLNNESSVVMGDFEKKATKTONINAY
Enterococcus phage vB_IME195 187 MKTGCSIAACGIMLEAGGLKPKDOLKTTFDYKHYHQENMDEBAQKVAVDLIVQL EEEETFYQNDITLSSLNNESSVVMGDFEKKATKTONINAY
Streptococcus phage C1 175 EKTNVSEIECGIMLNLNGLDEQYIKTETFYKVDLDTDWHVEVRYREFVNDLDEKQRYVYRNDVITLALTKHISSEFEGDEBEKQTFQNIKEEY
Staphylococcus phage P68 169 MKTNTSIAATLCEGLMGLGGLTESOLKTNNTITDKDNDMNSSEAYDYKCKAKLPEOLYVYRNDVITLALTKHISSEFEGDEBEKQTFQNIKEEY
Streptococcus phage Cp-1 120 KILNFSIATMGLF-----KMPIAKCTPLLR-----H-----KPEWIDYVYRNDVITLARGIFA---VYEEENFKVTSASE---AL
Bacillus phage phi 29 121 KKLPPFKKIDRF-----KLTVLKGDIDYHK-----P-----RPIGKIPPEEYVYRNDVITLAEAL---IQKQGLDMTAGSD---SL
Actinomyces phage Av1 137 KKLPMVSAIKAF-----NLHDQGLEIDYEK-----P-----RPIGKIPPEEYVYRNDVITLAEAL---IQKQGLDMTAGSD---SL

Motif CT Motif A or 1

Enterococcus phage vB_Efae230P-4 287 TI--NNARQLGKQITREGVKTISINYDYSNGENFATIQKIFYGGLNLYNCKYAKLITHEMSPDINSYVPSMYEFAFALHVIDYCEKET-
Enterococcus phage vB_IME195 287 TI--NNARQLGKQITREGVKTISINYDYSNGENFATIQKIFYGGLNLYNCKYAKLITHEMSPDINSYVPSMYEFAFALHVIDYCEKET-
Streptococcus phage C1 275 ANINDAREQLKQIGD---NMTGRHRLDYFQCCNAYDYFNNYGGNLYNCKYAKLITHEMSPDINSYVPSMYEFAFALHVIDYCEKET-
Staphylococcus phage P68 269 LN--NE--TRQILNQYQD---IKTSYHYHFFHDMNFYDYFSEFYGGNLYNCKYAKLITHEMSPDINSYVPSMYEFAFALHVIDYCEKET-
Streptococcus phage Cp-1 191 EKKRFRF-----KSKRFRDFPFLDERVDDFCRHIVYVGLRPLTKHRGTINQLDIDINSYVPSMYEFAFALHVIDYCEKET-
Bacillus phage phi 29 193 KGLKDIIT-----TKKFKVPTLSLGLDLYRYAVYVGGSETLNDP-FKEIKEIGVYVYRNDVITLAEAL---IQKQGLDMTAGSD---SL
Actinomyces phage Av1 209 ATPKK--T-----GATFIRRPFLSPEIDTETKAYVGGTADP--YAKKNGKGSVYDINSYVPSMYEFAFALHVIDYCEKET-

phi 29 TPR-1

Enterococcus phage vB_Efae230P-4 385 ---NINTEEDKRFILYRVKKT-FN-R---HSCDIDRVGR---QMIVKVRIV-EDSDVYLTSTFRMLKENFNINIELEVEQYKFSVFPGGIKK
Enterococcus phage vB_IME195 385 ---NINTEEDKRFILYRVKKT-FN-R---HSCDIDRVGR---QMIVKVRIV-EDSDVYLTSTFRMLKENFNINIELEVEQYKFSVFPGGIKK
Streptococcus phage C1 385 ---VDINTEEDKRFILYRVKKT-FN-R---HSCDIDRVGR---QMIVKVRIV-EDSDVYLTSTFRMLKENFNINIELEVEQYKFSVFPGGIKK
Staphylococcus phage P68 370 LKNIGSTGDYMFENLMED-VNDQ---HSDRISNVI---SALVVKVRK---IGYVILNVMVLSLIEITIKHQFNNLHVQSSVVECEHFGKRIE
Staphylococcus phage Cp-1 360 LPTFLDLDNYSLEIDKRVFNDD---LKKRVRVR---QMIVKVINND---NLYVNTNTTIRMIOQLITGDICMIRVNSVILECEYFHEBRIE
Bacillus phage phi 29 275 ---EIKDLY---HYIKADFLLKRYLPTQIKKKIDALRIGVRRSDVVTISKNVEVDIYLTNDDIDFLKHGDTI---MYVETLEFQTESD
Actinomyces phage Av1 274 ---WDLYPLH---QIICSEFELKEGYIPTQIKRSRF---YKGNELKSGSGGELADMLSNVDLEMLKHEHDLNVN---EYI--GLKFKKTTG
289 -----TNRPL---ASITFPAKLPNHPICQIKKNS---FNPTQYLEEK-PTTVAIIDELEW-KHYDEKLY-----SWNGTFEERSHGF

Motif B or 2a phi 29 TPR-2

Enterococcus phage vB_Efae230P-4 274 VDFYTKTQGRSKTLTEFKD--NNFTNIFETDR---PSKVFVRPEVIDISKVNLNGYVGPALRPT---YSGYVRDDNQLLERNFENTER-NALISVFT
Enterococcus phage vB_IME195 474 IDFYTKTQGRSKTLTEFKD--NNFTKIFETDR---PSEVFRPEVIDISKVNLNGYVGPALRPT---YSGYVRDDNQLLERNFENTER-NALISVFT
Enterococcus phage C1 474 IDFYTKTQGRSKTLTEFKD--NNFTKIFETDR---PSEVFRPEVIDISKVNLNGYVGPALRPT---YSGYVRDDNQLLERNFENTER-NALISVFT
Staphylococcus phage P68 460 AKNYIKTQGRMSKALNCTMETIDELNPLDDDKPKPEYDFHEMVEGSKVNLNGYVGPALRAY---DCYRRDNGQLYVNSFENTER-NIFSGAVY
Staphylococcus phage Cp-1 450 RQNYIKTQGRSKLNKNNMST---DYDHIIDD---INEIPSNBEMLSKVNLNGYVGPALRSH---NIFRDLNNEPLNINIENTER-NIFSGAVY
Bacillus phage phi 29 362 RPYITIVY-----RY-----RKENAQPAEKQKAKMLNSLYGKFAALLISVKKIVLDKGLIFKNDDSEVQPVYVPAVAFI
Actinomyces phage Av1 357 EKDIDW-----KTISE-GAIKQAHMLNSLYGKFAALLISVKKIVLDKGLIFKNDDSEVQPVYVPAVAFI
369 ---YVDHF-----ME-----TKNST-GGLRQLAMHLSNGKFAALLISVKKIVLDKGLIFKNDDSEVQPVYVPAVAFI

Motif 2b Motif C Motif 4

Enterococcus phage vB_Efae230P-4 567 TNGALVRLTQPPRYLGRBIDKQVYCDTDSLVMIRKCFKLPKPMHFANLGSWD EHEITIKFVYLNHKKYAYYA---EN-----BIQ
Enterococcus phage vB_IME195 567 TNGALVRLTQPPRYLGRBIDKQVYCDTDSLVMIRKCFKLPKPMHFANLGSWD EHEITIKFVYLNHKKYAYYA---EN-----BIQ
Streptococcus phage C1 557 TNGALVRLTQPPRYLGRBIDKQVYCDTDSLVMIRKCFKLPKPMHFANLGSWD EHEITIKFVYLNHKKYAYYA---EN-----BIQ
Staphylococcus phage P68 541 TNRSLNLLVPEQVLESIDDDNFTCDTDSLVMIRKCFKLPKPMHFANLGSWD EHEITIKFVYLNHKKYAYYA---EN-----BIQ
Streptococcus phage Cp-1 437 TSHARHFTS---NAGCNDNFTCDTDSLVMIRKCFKLPKPMHFANLGSWD EHEITIKFVYLNHKKYAYYA---EN-----BIQ
Bacillus phage phi 29 431 TSHARHTTIT---AQCAYERTICDTSLEHTGETEPEIRKIVDVKKLGVAHSTFKRKY---RCRMIQDLYMKRVDGKLVGSPDDYDTLDFKS
Actinomyces phage Av1 442 TSHARHTTIS---AQCNYERTICDTSLEHTGETEPEIRKIVDVKKLGVAHSTFKRKY---RAKQYABEG---G-----KID

Enterococcus phage vB_Efae230P-4 649 FRCGCPFLS---FDNNMFERFETQFSGAKANKRILYTHESTVVIYDSVTELDGNTYPEYLFHDEKEFSK-----IDLARELKDDEDS
Enterococcus phage vB_IME195 649 FRCGCPFLS---FDNNMFERFETQFSGAKANKRILYTHESTVVIYDSVTELDGNTYPEYLFHDEKEFSK-----IDLARELKDDEDS
Streptococcus phage C1 641 VRCGCSKALIKKWIARSNRNIIYFNNFIDGVTPATRIKREWNTITVYDTEBLKCGEVYKRYDTNLLQNIESLAKLKDALTTESETSLDYS
Staphylococcus phage P68 623 TASSGCPKNA---PDTSDFFETREDFDGALENNNSIYEQCTSIYSEKTEVCGNVYDENFTDLNMKREFLKD---ARENFDSQFDD
Streptococcus phage Cp-1 515 VRCGCPFLS---FDNNMFERFETQFSGAKANKRILYTHESTVVIYDSVTELDGNTYPEYLFHDEKEFSK-----IDLARELKDDEDS
Bacillus phage phi 29 525 VRCGCPFLS---FDNNMFERFETQFSGAKANKRILYTHESTVVIYDSVTELDGNTYPEYLFHDEKEFSK-----IDLARELKDDEDS
Actinomyces phage Av1 517 VHIHGFRNVAATL---LED---MLGGHWNGLLIPVRVGGVVKDITFTLID-----

Enterococcus phage vB_Efae230P-4 738 VLYEESDGLKADLQYEBEBSKDIWDVVDSDREINCLIBN---
Enterococcus phage vB_IME195 738 VLYEESDGLKADLQYEBEBSKDIWDVVDSDREINCLIBN---
Streptococcus phage C1 741 TLYEESNIGFESDLKIKKNNIKOSSMVD---BYDFKSYLIY
Staphylococcus phage P68 712 VLYEESDGFSLNDLIPVRSVHNSDLDLHK---REHDEKKGNC
Streptococcus phage Cp-1
Bacillus phage phi 29
Actinomyces phage Av1

Figure S1: Structural and functional map of family of B phage DNA polymerases belonging to the “protein priming” subfamily.

Alignments of the residues of *Enterococcus* phage VB_Efae230P-4, *Enterococcus* phage Idefix, *Enterococcus* phage VB_IME195, *Streptococcus* phage C1, *Staphylococcus* phage P68, *Streptococcus* phage Cp-1, *Bacillus* phage phi 29 and *Actinomyces* phage Av1 DNA polymerases are shown. Three N-terminally located motifs ExoI, ExoII and ExoIII (marked in red) constitute the 3'-5' exonuclease active site which is conserved in eukaryotic and prokaryotic polymerases [67,70]. The motif ExoII additionally presents 3 conserved residues (marked in orange) involved in the interaction with the Terminal Protein [69]. The N-terminal domain is separated from the C-terminal domain by a cross-talk conserved motif CT (marked in blue) playing an important role in the coordination between the previously mentioned proofreading activity and the synthesis activity performed in the C-terminal domain. Three C-terminally consensus motifs A, B, and C (marked in dark green) form the polymerase active site that is conserved in the other polymerases from the B family [67,70]. Two additional motifs 2b and 4 (marked in light green) notably involved in the primer stabilization are found in several B DNA polymerases. The subfamily of protein-priming DNA polymerases specifically harbor two inserted sequences TPR-1 and TPR-2, interspersed between conserved motifs A and B and B and C respectively. These 2 sequences have notably been studied in phi 29 DNA polymerase (marked in purple) and demonstrated as involved in Terminal Protein interaction and both strand displacement and processivity respectively [68,71]. Whether the 2 inserted sequences are not conserved among the protein priming subfamily of B DNA polymerase, several identical residues (underlined in yellow) are found in TPR-1 [68].

A

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amidase Lactobacillus kimchicus JCM 15530 1 MAGEVSRLLTSVNFNMMNAGSRNGIPIIDTIIHHNATTNKDVAAMTWLGGGAGTSAHYECTPETEIIIGCVGEQVSAFHAGGTGGSDVPTKIANPNORSIG
amidase Enterococcus phage EFDG1 1 MAGEVSRLLTSVNFNMMNAGSRNGIPIIDTIIHHNATTNKDVAAMTWLGGGAGTSAHYECTPETEIIIGCVGEQVSAFHAGGTGGSDVPTKIANPNORSIG
amidase Enterococcus phage phiEF24C 1 MAGEVSRLLTSVNFNMMNAGSRNGIPIIDTIIHHNATTNKDVAAMTWLGGGAGTSAHYECTPETEIIIGCVGEQVSAFHAGGTGGSDVPTKIANPNORSIG
amidase Enterococcus phage EFLK1 1 MAGEVSRLLTSVNFNMMNAGSRNGIPIIDTIIHHNATTNKDVAAMTWLGGGAGTSAHYECTPETEIIIGCVGEQVSAFHAGGTGGSDVPTKIANPNORSIG
IDF_15 Enterococcus Phage Idefix 1 --MAGEVSRLLTSVNFNMMNAGSRNGIPIIDTIIHHNATTNKDVAAMTWLGGGAGTSAHYECTPETEIIIGCVGEQVSAFHAGGTGGSDVPTKIANPNORSIG

amidase Lactobacillus kimchicus JCM 15530 101 FENVNSGAPNWSYDPPTTNCARLVADICRKYGTPCDRQHVLGHNVTATACPGGMDVDEVRQAKSPMAGGNSSTIATFRQAFEDVNVN---SAGV
amidase Enterococcus phage EFDG1 101 FENVNSGAPNWSYDPPTTNCARLVADICRKYGTPCDRQHVLGHNVTATACPGGMDVDEVRQAKSPMAGGNSSTIATFRQAFEDVNVN---SAGV
amidase Enterococcus phage phiEF24C 101 FENVNSGAPNWSYDPPTTNCARLVADICRKYGTPCDRQHVLGHNVTATACPGGMDVDEVRQAKSPMAGGNSSTIATFRQAFEDVNVN---SAGV
amidase Enterococcus phage EFLK1 98 FENVNSGAPNWSYDPPTTNCARLVADICRKYGTPCDRQHVLGHNVTATACPGGMDVDEVRQAKSPMAGGNSSTIATFRQAFEDVNVN---SAGV
IDF_15 Enterococcus Phage Idefix 98 FENVNSGAPNWSYDPPTTNCARLVADICRKYGTPCDRQHVLGHNVTATACPGGMDVDEVRQAKSPMAGGNSSTIATFRQAFEDVNVN---SAGV

amidase Lactobacillus kimchicus JCM 15530 197 DGFQKQALRFVKVTR-----LAEPEKYG-----LTFPK-----LISGLGSSNKAFL-----KFTLA
amidase Enterococcus phage EFDG1 197 NQCGPISLTKKQDS-----LYPMDKKT-----LTFPK-----LISGLGSSNKAFL-----KFTLA
amidase Enterococcus phage phiEF24C 201 LCLPESNSKTYEINGDAMVMECNGVNC-----VHPDEM-----EDVHL--KNNGDDIPVYQ-----GE
amidase Enterococcus phage EFLK1 201 LCLPESNSKTYEINGDAMVMECNGVNC-----VHPDEM-----EDVHL--KNNGDDIPVYQ-----GE
IDF_15 Enterococcus Phage Idefix 191 TYM--K-----QKNGNTEQWFCGNKRMVLPV-----NANAIRYGGSSNQTVINHDNFGKLEKAT-----

amidase Lactobacillus kimchicus JCM 15530 247 ID-----
amidase Enterococcus phage EFDG1 275 LNDNKEELVTDKVKAPALNRGLDVIKGDGQVTLHGHWLSSKRRNSDKHFLFIMDKKTNKEIVRFDVTKSSFFKASPDIQKLVDTIAQRNCREA
amidase Enterococcus phage phiEF24C 266 NKN--APV--NR-----ETV--PVVGIITKSS-----
amidase Enterococcus phage EFLK1 266 NKN--APV--NR-----ETV--PVVGIITKSS-----
IDF_15 Enterococcus Phage Idefix 255 -----EVKV-----

amidase Lactobacillus kimchicus JCM 15530 -----
amidase Enterococcus phage EFDG1 375 FAHALDAKSPARGKDIYILSRVCSDPAGNNGISDQLQGGTHKL-----
amidase Enterococcus phage phiEF24C -----
amidase Enterococcus phage EFLK1 -----
IDF_15 Enterococcus Phage Idefix -----
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B

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amidase Enterococcus phage SAP6 1 -----MVKNDVLYVNGLVGKVDADGWYGTQCMD-----LTVDMQRFFGWRFRYGNALIALVDQPLPAGFORIR
lysin Enterococcus phage IMEEF1 1 -----MVKNDVLYVNGLVGKVDADGWYGTQCMD-----LTVDMQRFFGWRFRYGNALIALVDQPLPAGFORIR
amidase Streptococcus phage SPQS1 1 -----MVKNDVLYVNGLVGKVDADGWYGTQCMD-----LTVDMQRFFGWRFRYGNALIALVDQPLPAGFORIR
IDF_15 Enterococcus Phage Idefix 1 --METYSKLTTSVNFNMMNAGSRNGIPIIDTIIHHNATTNKDVAAMTWLGGGAGTSAHYECTPETEIIIGCVGEQVSAFHAGGTGGSDVPTKIANPNORSIG

amidase Enterococcus phage SAP6 66 FTSSIQKAGDWMWGLGYAAYGHTGIATEDGRDCTFVSDQNWNPVLEVGSPAAAHHNMDGVGCV-----IRPPEEAASTPKPPAPKPKDKPNLQGV
lysin Enterococcus phage IMEEF1 66 FTSSIQKAGDWMWGLGYAAYGHTGIATEDGRDCTFVSDQNWNPVLEVGSPAAAHHNMDGVGCV-----IRPPEEAASTPKPPAPKPKDKPNLQGV
amidase Streptococcus phage SPQS1 66 FTSSIQKAGDWMWGLGYAAYGHTGIATEDGRDCTFVSDQNWNPVLEVGSPAAAHHNMDGVGCV-----IRPPEEAASTPKPPAPKPKDKPNLQGV
IDF_15 Enterococcus Phage Idefix 95 IGLEHNSGGAPSCISDDTLRNSAKLIAICQ--RYGPIINRPIKAHNEVYATASPGGINDKRIVRAQDAANGK-----CDEPKKPLPKPKKEDD

amidase Enterococcus phage SAP6 162 KGGDDIMFIYKTKQGS-----TEQWVFIGGKRIYLPMTYVNEANDLIKRYGGTNVTVYNHDFLKMMEALPOVKV-----
lysin Enterococcus phage IMEEF1 162 KGGDDIMFIYKTKQGS-----TEQWVFIGGKRIYLPMTYVNEANDLIKRYGGTNVTVYNHDFLKMMEALPOVKV-----
amidase Streptococcus phage SPQS1 162 KGGDDIMFIYKTKQGS-----TEQWVFIGGKRIYLPMTYVNEANDLIKRYGGTNVTVYNHDFLKMMEALPOVKV-----
IDF_15 Enterococcus Phage Idefix 188 -----KFTYMKKCKLKN-----TEQWVFIGGKRIYLPMTYVNEANDLIKRYGGTNVTVYNHDFLKMMEALPOVKV-----
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Figure S2: IDF_15 alignments. (A) Alignments of residues of IDF_15 with amidases with similar N terminal domains identified by BLASTP. IDF_15 N-terminal domain (residues 21-153) is predicted to correspond to a N-acetylmuramoyl-L-alanine amidase domain on pfam (<http://pfam.xfam.org/>). IDF_15 shares ~ 60% amino acids identity and ~ 60-75% coverage (N terminal terminuses) with amidases encoded by a *Lactobacillus kimchicus* strain and *Enterococcus* phages EFDG1, phiEF24C and EFLK1. (B) Alignments of residues of IDF_15 with hydrolases with similar C terminal domains identified by BLASTP. IDF_15 C shares ~ 70 % amino acids identity and ~ 40 % coverage (C-terminuses) with amidases and lysin encoded by an *Enterococcus* phage SAP6, *Streptococcus* phage SPQS1 and *Enterococcus* phage IMEEF1 respectively.

A

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IDF_13 Enterococcus phage Idefix        1  MSYDITTEDYQALLSEEGYDFGSDETIAWFMFGGARPVNSYGVTKNLSDYIPKIQEFGGSLFLMPTVSEGGGAGNWNHMYSDTSNTG
PlyCA Streptococcus phage C1              1  --MSRRTVQGYEKLADQANNTFGSFOQLAFWFPGGARPVNSYGVNANLSDYIPKIQEYGVSYTFLMPTVSEGGGAGNWNHMYSDTSNTG

IDF_13 Enterococcus phage Idefix       101  GVIDDARTITRHRFPFASAPEVGGVTEDEELTMVVAEDGSESYGFSTMAGNAWFGSRTVQVNGAAPVVFNGFYDQLLIRKSF
PlyCA Streptococcus phage C1              99  ECSEHDQDIIHVWVTYFPFASAPEYFATPDNAAALDSEYSESRVSDVMTSTMAGNAWVAVVNGAAPVVFNGFYDSCDLSLHAW

IDF_13 Enterococcus phage Idefix       201  ADPPEEGTAKPNPTPK--DPNEANPKPKDPCKAKDKLDEINKADNQTIQGSPLLSNDVFIERTFNNYSKSLTSPFKHMLNASTG
PlyCA Streptococcus phage C1              197  ADPPTGSGITDGGKNSVYGTGNATVSSSEANPKKKAATDFNNLHESGSE-----FENQIEN-----AKVGLLCELQDGLNATQ

IDF_13 Enterococcus phage Idefix       299  LTRIDGAVTNEPKDINPP-----SIDGCKSKMFKIYDWCNNGCAAFDGDYGYGQVDSLSEINNTVPSLGLDTSGDYA-K-----NWNPPFSG
PlyCA Streptococcus phage C1              281  LRAIVNLTNENPKKFTVQSPGQDLSGSSSERVA--ANL-----ANLQAVQVKYIGDQCYAVVGMSAVYCSYSSVSGDPLPLIGDGNHSHLS

IDF_13 Enterococcus phage Idefix       388  MKVNGFNDDNASRDINWTLNPAIYVNSGCAHWVAGKDFATIQQNFVSHLGEPIVLADCASWVSSGSEEGAVTIDN-----
PlyCA Streptococcus phage C1              374  RHW-----SIANTHINLP---VFVGR---EDRIGALICATFSAFFYTG-----QYGHFESADTITVLEQNILGSPVIR

IDF_13 Enterococcus phage Idefix       447  -----
PlyCA Streptococcus phage C1              447  STYDLNTFLSTLTGLITFK

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B

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Peptidase M23 Lactobacillus reuteri 100-23  1  -----EFCKSGEINAKPSDIINMKDFAIRSS--SVRAQVDVNRLY---VWLNASSPPEWFFAHLIQGQ-TYYGQLNRHRHSDAR
Phage lysin Lactobacillus antri DSM 16041  1  -----SIVREARKSPINADFGVNKAQLDSFAACDH--RVAHAQVDVNRLY---DTRKQAGSPPEWFFADLQDPMNSNLRNHRNPRFP
Peptidase M23 Lactobacillus oris PB013-T2-3  1  -----SIVREARKSPINADFGVNKAQLDSFAACDH--RVAHAQVDVNRLY---DTRKQAGSPPEWFFADLQDPMNSNLRNHRNPRFP
PlyCA N ter Streptococcus phage C1          1  --MSKKYTYTTPYOKLACANFTGFSEFQADWFMFGGARPVNSYGVNANLSDYIPKIQEYGSPTLDMPTFSSG--GAGNANRHWYDTSNG
IDF_13 N ter Enterococcus phage Idefix    1  MPYKYTYTTPYOKLACANFTGFSEFQADWFMFGGARPVNSYGVTKNLSDYIPKIQEFGGSLVLMPTVSEGGGAGNWNHMYSDTSNTG

Peptidase M23 Lactobacillus reuteri 100-23  80  SDAQ--SFCSEKIKCSNENINPARSEGESI--F--P--P--DKWNOEFPKGTIGRVLQQTAAW/-----LAC--QTENPALFHS--SCTCFR
Phage lysin Lactobacillus antri DSM 16041  85  QDA--SVCWIKFKFANSPSEPSY--T--T--T--TSPQ--RAGNOEFPKGTIGRVLQQTAAW/-----LAN--E--R--R--R--R--R--R--R--R--R
Peptidase M23 Lactobacillus oris PB013-T2-3  84  QDA--SVCWIKFKFANSPSEPSY--T--T--T--TSPQ--RAGNOEFPKGTIGRVLQQTAAW/-----LAN--E--R--R--R--R--R--R--R--R--R
PlyCA N ter Streptococcus phage C1          98  LSCSEHDL--EGVWVTYFPFASAPEYFATPDNAAALDSEYSESRVSDVMTSTMAGNAWVAVVNGAAPVVFNGFYDSCDLSLHAW
IDF_13 N ter Enterococcus phage Idefix    100  MGCIDDIAIERT--TFDRHPEPALSPEYFATPDNAAALDSEYSESRVSDVMTSTMAGNAWVAVVNGAAPVVFNGFYDSCDLSLHAW

Peptidase M23 Lactobacillus reuteri 100-23  166  WGH--NAT--CGNGWPFDPVGEH-----
Phage lysin Lactobacillus antri DSM 16041  167  WGH--NS--GGSGWPBANIP-----
Peptidase M23 Lactobacillus oris PB013-T2-3  165  WGH--VKGCGGGWPFPCGEGQFALGQTFTGTHPDGVRGNG
PlyCA N ter Streptococcus phage C1          165  WGH--VKGCGGGWPFPCGEGQFALGQTFTGTHPDGVRGNG
IDF_13 N ter Enterococcus phage Idefix    199  RQIDPFEE--STI-----KPNPTKPKD-----

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C

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P60_LISMO autolysin Staphylococcus monocytogenes 380  -----L GKAYSFSGSP-----TIDSGTKVFAA--GSLRPF-----TSG--Q--AST-----TRIS
LYTE_BACSU autolysin Bacillus subtilis        231  -----VGTPLYKSGITTT-----TDSGTLWVNLN--Q--T-SVGR-----TST--G--WSS-----MKS
PlyCA C ter Streptococcus phage C1              309  GSRYV-----TLANAQGVKELGDCGDAVGVGWSAVTGYISYSTGDPMLPLIGDGMNHSIHLDWSIANTGIVVTFPTGVRKEDLRVA
IDF_13 C ter Enterococcus phage Idefix       323  R--SKTMKKIYDWCNNGCAAFDTGCTV--T--T--T--TSPQ--RAGNOEFPKGTIGRVLQQTAAW/-----LAN--E--R--R--R--R--R--R--R--R--R
056785_9CAUD hydrolase Staphylococcus aureus 187  14  -----LITGIGVNDVDF--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S
LYTA STAAU autolysin Staphylococcus aureus    18  -----LKTSEKGFNVLPLVY--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C--C
056788_9CAUD amidase Staphylococcus phage Twort 11  -----IKSKVN--GTDF--SL--D--Q--Q--LAVDYIY--TDCGRW--NAK-DA-----INNSFG--T--ATV-----KVP

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P60_LISMO autolysin Staphylococcus monocytogenes 427  ESQAHEGD--VFDY--S--IS--SH--CY--NG-----
LYTE_BACSU autolysin Bacillus subtilis        277  ASP-SVDFP--E--YK--S--PS--CY--SN-----
PlyCA C ter Streptococcus phage C1              402  I-CAT-----AGAPPLYTGG--CH--E--SWSDTT-VLEQNILGSPVIRSTYDNLNTFLSTLTGLITFK
IDF_13 C ter Enterococcus phage Idefix       404  I-NLT--NGA--F--NS--R--H--K--K--K--G--K--K--G--F--A--M--L--Q--N--T--S--H--L--G--G--P--I--V--L--A--D--C--A--S--W--M--V--S--S--G--S--G--L--G--A--W--I--D--N
056785_9CAUD hydrolase Staphylococcus aureus 187  76  D-VPK--SD--A--M--G--G--N--Y--N--T--S--R--S--I--P--S-----
LYTA STAAU autolysin Staphylococcus aureus    74  D-LAQ--SD--V--S--NYG--A--G--H--W--EAT-----
056788_9CAUD amidase Staphylococcus phage Twort  71  A-RPKYED--V--H--G--N--F--A--T--G--H--T--N--P-----

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Figure S3: Alignments of IDF_13. (A) Alignment of the residues of IDF_13 and those of the catalytic subunit PlyCA encoded by *Streptococcus* phage C1. BLASTP indicates that the two proteins share 37 % amino acids identity and 77 % coverage. (B) Alignments of the residues of IDF_13 N-terminal domain with glycosyl hydrolases with similar domains identified by PSI-BLAST (modified from [75]). PlyCA N terminal domain (residues 1-205) corresponds to a glycosyl hydrolase domain homologous to those found in peptidases M23 and phage lysin encoded by *Lactobacillus reuteri*, *oris* and *antri* respectively. IDF_13 N-terminal domain shares 55 % amino acids identity and 97 % coverage with PlyCA N terminal domain, it notably harbors two out of the three conserved residues (marked in red) which are supposed to represent a catalytic center [75]. (C) Alignments of the residues of IDF_13 C-terminal domain with CHAP domains identified by PSI-BLAST (modified from [74,76]). PlyCA C-terminal domain (residues 309 to 465) corresponds to a CHAP domain with an *N*-acetylmuramoyl-L-alanine amidase activity [75]. The C-terminuses of IDF_13 and PlyCA are less conserved than the N-terminuses. Nevertheless, analyses reveal that IDF_13 C-terminal domain shares similarity with CHAP domains identified in autolysins encoded by *Staphylococcus monocytogenes*, *Staphylococcus aureus* and *Bacillus subtilis* or amidase and hydrolase encoded by *Staphylococcus* phages Twort and 187 respectively. IDF_13 C-terminal domain harbors the two predicted catalytic residues (marked in orange) which characterized CHAP domains [74,76].

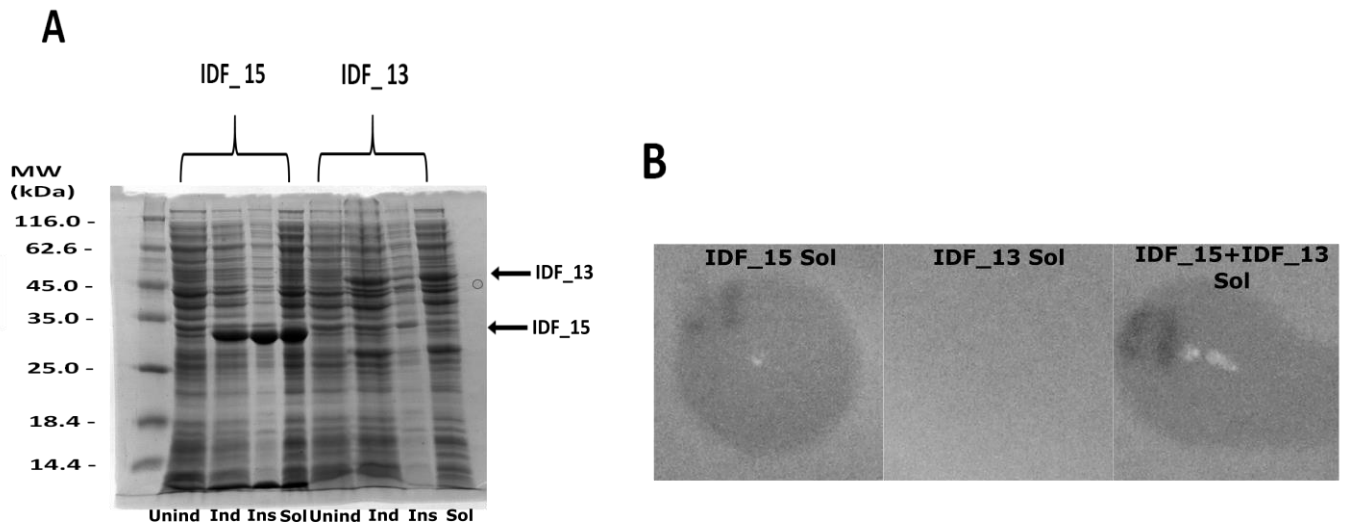


Figure S4 : Activities of *E.coli* extracts containing putative Idefix endolysins (IDF_15 and IDF_13). (A) Production of IDF_15 (29.6 kDa) and IDF13 (52.2 kDa) in *E. coli* ER2566 transformed by pAB2 and pAB1 respectively were assessed by analysis of 10 μ L of harvested induced (ind) or uninduced (unind) cells resuspended in lysis buffer (10X concentration compared to the cell culture). After lysis and centrifugation, 2 μ L of soluble (sol) and insoluble (insol) extracts (50X concentration compared to the cell culture) of uninduced or induced cells were analyzed by SDS-PAGE (12.5%) and coomassie blue staining, revealing an induced protein in the soluble fraction, of a size compatible with IDF_15 and IDF_13, respectively. (B) Evaluation of IDF_15 and IDF13 activities on pp, 5 μ L of soluble extracts of induced cells containing IDF_15 or IDF13 and 5 μ L of a mix of the two previous fractions were respectively spotted on a pp bacterial lawn.

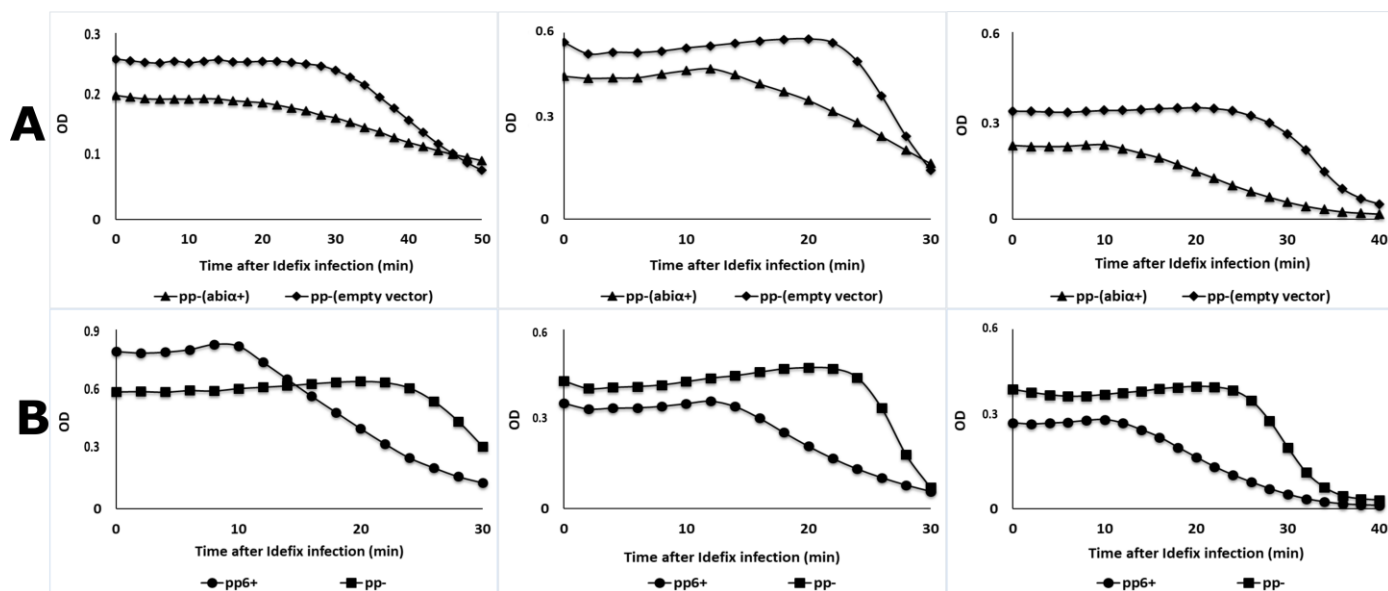


Figure S5 : Biological replicates relative to lysis curves experiments after Idefix infection at MOI 10. (A) Comparison between pp-pJIM2246abiα⁺ (VEJL5) and pp-pJIM2246 (VEJL4) in triangles and diamonds, respectively (three independent experiments). **(B)** Comparison between pp6⁺ and pp⁻ in circles and squares, respectively (three independent experiments).

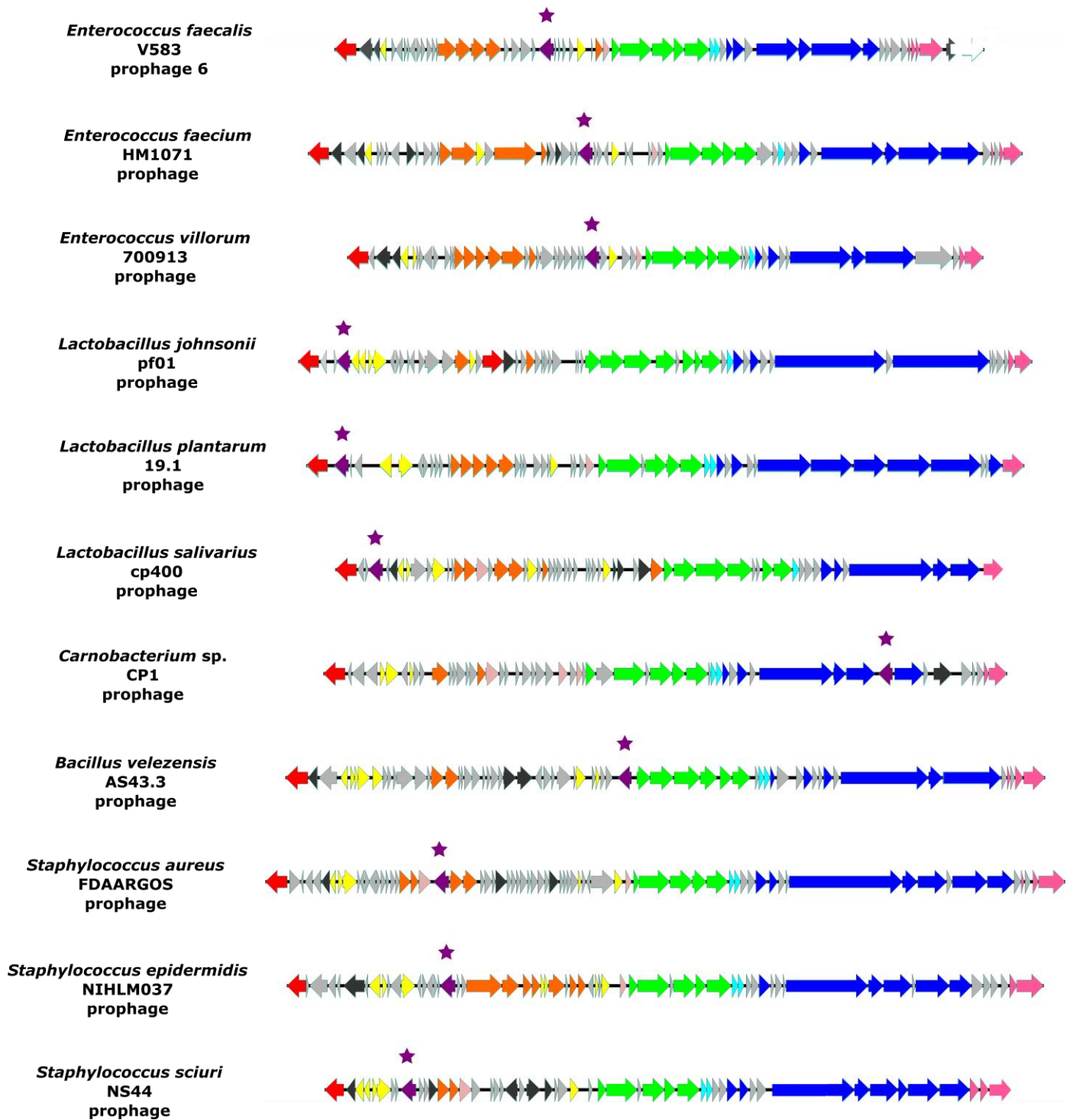


Figure S6: Putative *abiax* homologs identified on several prophages of Gram-positive bacteria. *abiax* and its putative homologs are colored in purple and marked with stars. Other gene functions are color-coded (red: integrase, yellow: transcriptional regulation, orange: DNA metabolism, green: DNA packaging and head, light blue: head to tail, dark blue: tail, light pink: HNH endonuclease, dark pink: lysis, grey: hypothetical proteins, white: transposable element, black: additional functions including putative anti-phage systems).

Table S1: Bacterial strains, plasmids and oligonucleotide primers used

Strain, plasmid or primer	Relevant characteristics or primer sequences	Reference or source
<u>Strain</u>		
<i>E. coli</i>		
JM105	<i>endA1 glnV44 sbcB15 rpsL thi-1 Δ(lac-proAB) [F' traD36 proAB⁺ lacI^q lacZΔM15] hsdR4(rk mk⁺)</i>	NEB
ER2566	F ⁻ <i>λ- fhuA2 ompT lacZ::T7pol gal sulA11 Δ(mcrC-mrr)114 mcr-73 zgb-210 endA1</i>	NEB
<i>L. lactis</i>		
MG1653	Genetically amenable plasmid-free strain	[46]
<i>E. faecalis</i>		
VE14002	V583 vancomycin resistant clinical isolate	[50]
VE14089	V583 vancomycin resistant clinical isolate cured of its plasmids	[48]
VE18590	VE14089 derivative deleted from all its plasmids and active prophages	[43]
VE18562	VE14089 derivative deleted from all its plasmids but pp1	[43]
VE18584	VE14089 derivative deleted from all its plasmids but pp3	[43]
VE18583	VE14089 derivative deleted from all its plasmids but pp3 and pp5	[43]
VE18582	VE14089 derivative deleted from all its plasmids but pp4	[43]
VE18589	VE14089 derivative deleted from all its plasmids but pp7	[43]
VE18581	VE14089 derivative deleted from all its plasmids but pp6	[43]
VE18306	VE14089 derivative only deleted from pp6	[43]
VEJL1	VE18306 derivative complemented with pJL1	This study
VEJL2	VE18306 derivative complemented with pJL2	This study
VEJL3	VE18306 derivative complemented with pJL3	This study
VEJL4	VE18590 derivative complemented with pJIM2246	This study
VEJL5	VE18590 derivative complemented with pJL3	This study
VE18393	VE18306 derivative deleted from <i>epaX</i> (<i>ef2170</i>)	This study
VE18944	VE18393 derivative complemented with pVE14176	This study
VE18945	VE18393 derivative complemented with pVE14297	This study
<u>Plasmid</u>		
pJIM2246	<i>E. coli</i> -Gram positive shuttle vector Cm ^r	[47]
pJL1	<i>ef2850</i> cloned in pJIM2246	This study
pJL2	<i>ef2847</i> cloned in pJIM2246	This study
pJL3	<i>ef2833</i> cloned in pJIM2246	This study
pVE14283	encompassing 5' and 3' ends <i>epaX</i> insert cloned in pG+host9	[49]
pVE14176	promoter PaphA3 vector made of pIL253 and pVE14041	[45]
pVE14297	<i>epaX</i> cloned downstream promoter PaphA3 in pVE14176	[49]
pJ411	PBR322 origin, kanR, pT7	Menlo Park CA
pAB1	<i>IDF_13</i> cloned in pJ411	This study
pAB2	<i>IDF_15</i> cloned in pJ411	This study
<u>Primer</u>		
JL12	TACGGATCCTTGAACAGCGGAACG	This study
JL13	AATGAGCTAGGGAGTGGAGTTTG	This study
JL10	TACGGATCCCTATCACCTCCTATAC	This study
JL11	TACGGATCCTTATCAAAG	This study
JL8	TACGGATCCTTAGCGCTGCCCTTAC	This study
JL9	AATGTCGACTG GCGAGTGACTGG	This study
JL20	ATGCGCGCGTTGTTTTAAATAATTGGAG	This study
JL21	ATCGCATGCGCATGATATTACTCTCCTAC	This study
OEF879	GGATTGGATTAGTTCTTGTG	Furlan et al, submitted
1233	AGCGGATAACAATTTACACAGGA	NEB
OEF528	TCATTCCTTCTCCCAAGCTTC	[48]
OEF880	GCGACAATGATTGTATTGTC	This study
OEF394	AGTTATCGGAGATGTCACAG	[49]
OEF397	ATCACCATTCACAAACCAC	[49]
OEF527	TGCTCGTAATGCTGGAATTG	[48]
OEF856	CTTTATTCGTTTCAAAAGTGG	Furlan et al., submitted
OEF823	TAGGGCCCAAGTAATGTTGATGAGAACG	Furlan et al., submitted
OEF859	AATTATCGGTTTTCTCTACC	Furlan et al., submitted
OEF857	AAATCTCGTCTCCTAATGC	Furlan et al., submitted
OEF885	TACACGTATTTACAGGATTGG	This study
OEF858	ATCTATACGCATCTGAAATGG	Furlan et al., submitted
OFL264	AATTCATATGCCTAGTTACAAAACCTTATAC	This study
OFL265	AATTGGATCCCTAATTATCTGTAAACCAAG	This study
OFL266	AATTCATATGGAAACCTACTCAAAATTAACAAC	This study
OFL267	AATTGGATCCTTATACTTAACTTCCGTATAAG	This study

Table S2. Description of natural *Enterococcus* isolates tested as potential Idefix hosts

<i>E. faecalis</i>						
Isolate (Other name)	Origin ^a	Source; origin; year of isolation	Ab ^{Rb}	ST ^c	CPS ^d	CRISPRs ^e
VE14001 (OG1RF)	C	Mouth; USA; 1978	F; R	1	T1	+/+
VE14002 (V583)	C	Blood; Missouri, USA; 1987	E; G; K; V	6	T2	-/+
VE14000 (JH2-2)	C	Hammersmith, UK; 1973	F; R	8	T2	-/+
VE14568 (G51)	C	Endocarditis; USA; <1992	M; T	19	T1	+/+
VE14569 (G52)	C	Endocarditis; USA; <1992	E; K; M; T	19	T1	+/+
VE14571 (G54)	Cm	Human stool; Oklaoma, USA; 1994	M; T	19	T1	+/+
VE14840 (F11/KA1.2) ^f	F	Cheese; France; 2005	C; E; K; M; T	21	T1	-/+
VE14741 (efs2)	F	Cheese (St Paulin); France; 1991	M; T	72	T5	-/+
VE14633 (1085)	F	Cheese (Salers); Cantal, France; 1991	-	277	T2	-/+
VE14578 (G61)	Cm	Human stool; Oklaoma, USA; 1994	M; T	275	T2	-/+
VE14794 (CE22)	F	Coat Cheese; Corse, France; 1982	-	279	T5	-/+
VE14820	C	Endocarditis; France	E; G; K; S	281	T5	-/+
VE18245 (HH22)	C	Urine; Houston, USA; 1981	A; G; E; T	6	T2	-/+ ^g
MMH594	C	Blood; Wisconsin, USA; 1985	E; G; K; S	6	T2	-/+
VE14937 (Symbioflor 1)	P*	Healthy human, Germany, 1950s	-	248	T1	-/+ ^g
VE14505	-	Unknown ^h ; <1950	-	25	T1	-/+
VE14513	C	Urine; Loir-et-Cher, France	-	168	T2	-/+
VE14518	C	Deep pus; Ardèche, France	-	145	T1	+/+
VE14565	C	Endocarditis; USA; <1992	M; T	55	T1	+/+
VE14573	Cm	Human stool; Oklahoma, USA; 1994	M; T	274	T1	+/+
VE14583	C	Lung; Bouches du Rhône, France	C; E; M; T	276	T1	+/+
VE14596	C	Valve's body; Rhône, France; 1999	M; T	40	T1	+/+
VE14605	S	Human stool; Yvelines, France; 2004	-	86	T5	-/+
VE14668	C	Urine; Yvelines, France	C; E; G; K; M; S; T	6	T2	-/+
VE14675	C	Deep Pus; Yvelines, France	C; E; G; K; M; T	159	T1	-/+
VE14724	Cm	Human stool; Doubs, France; 2003	-	278	T1	+/+
VE14726	Cm	Human stool; Doubs, France; 2003	-	206	T2	-/+
VE14807	Cm	Human stool; Yvelines, France; 2005	-	35	T2	+/+
VE14816	C	Endocarditis; France	E; G; K; M; T	16	T2	+/+
VE14817	C	Endocarditis; France	C; K; M; T	280	T2	+/+
VE14818	C	Endocarditis; France	-	97	T1	-/+

VE14821	C	Endocarditis; France	-	209	T5	-/+
VE14822	C	Endocarditis; France	-	282	T5	+/+
VE14824	F	Cheese; France; 2005	E; M; T	21	T1	+/+
VE14828 ^f	F	Cheese; France; 2005	C; E; K; M; T	283	T1	-/+
VE14842 ^f	F	Cheese; France; 2005	E; K; M; T	23	T2	-/+
VE14843 ^f	F	Cheese; France; 2005	K; M; T	133	T1	-/+
VE14844 ^f	F	Cheese; France; 2005	C; E; K; M; T	284	T1	+/+
VE14845 ^f	F	Cheese; France; 2005	C; E; M; T	285	T1	+/+
VE14870 ^f	F	Cheese; France; 2005	K; M; T	9	T5	-/+
VE14878 ^f	F	Cheese; France; 2005	E; K; M; T	26	T1	-/+
VE14893	C	Endocarditis; Doubs; France; 2004	-	286	T2	+/+
VE14927	C	Endocarditis; Ile et vilaine, France	E	288	T1	-/+
VE14929	C	Endocarditis; Ile et vilaine, France	C; E; K; M; S; T	41	T1	-/+
Ef230 ⁱ	S	Urban sewage, Poland	V	nd	nd	nd
Ef423 ⁱ	S	Urban sewage, Poland	V	nd	nd	nd
Ef546 ⁱ	S	Urban sewage, Poland	-	nd	nd	nd
<i>E. faecium</i>						
VE14727 (BM4147) ^j	C	Patient stool; France; 1986	V;T	95/CC22	nd	nd
VE14141 (TX0016) ^j	C	Blood from endocarditis patient ; Houston; 1992	K; E; Sm, T	18	nd	nd
VE14976 (06-007) ^j	C	French national reference center; France; 2006	Va	21	nd	nd
VE14977 (06-046) ^j	C	French national reference center; France; 2006	Va	64	nd	nd
VE14978 (06-047) ^j	C	French national reference center; France; 2006	Va	78	nd	nd
VE14980 (06-084) ^j	C	French national reference center; France; 2006	Va	18	nd	nd
VE14981 (07-018) ^j	C	French national reference center; France; 2007	Vb	203	nd	nd
VE14982 (07-079) ^j	C	French national reference center; France; 2007	Vb	202	nd	nd
VE14983 (07-095) ^j	C	French national reference center; France; 2007	Va	17	nd	nd
VE14984 (08-225) ^j	C	French national reference center; France; 2008	Vb	192	nd	nd
VE14988 (09-122) ^j	C	French national reference center; France; 2009	Va	280	nd	nd
VE14989 (Aus0004) ^j	C	French national reference center; Australia; 1998	Vb	17	nd	nd

a: C, Cm, F, and S correspond to clinical, commensal, food and sewage origin, respectively. P* indicates one isolated colony from a probiotic concoction of ten *E. faecalis* isolates. – indicates unknown origin. b: A, C, E, F, G, K, M, R, S, Sm, T and V indicate resistance to ampicillin, cotrimoxazole, erythromycin, fusidic acid, gentamicin, kanamycin, minocycline, rifampicin, sulfamethoxazole/trimethoprim, streptomycin, tetracycline and vancomycin, respectively. Va and Vb indicate the gene involved in vancomycin-resistance. c: Sequence Type. nd indicates not documented. d: Capsule Type. nd indicates not documented. e: CRISPRs indicates the CRISPR1/CRISPR2-group assigned from PCR CRISPR-specific amplification. – indicates no detection. nd indicates not documented. f: Isolates for which some details have been ever given in [52]. g: CRISPRs data were obtained using [24,51]. h: The origin of the type strain is contradictory in the literature. i: Isolates graciously provided by Dr. Sylwia Bloch and Dr. Alicja Węgrzyn from Department of Molecular Biology, and University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland. These strains allows the propagation of podophage vB_Efae230P-4 [44] homologous to Idefix. j: Isolates initially graciously provided by Pr. Vincent Cattoir from CNR souches Enterocoques, Rennes, France.

Table S3: Respective genome accession numbers of the *Enterococcus Picovirinae* phages

<i>Enterococcus Picovirinae</i> phages	Genome accession number
<i>Enterococcus faecalis</i> phage Idefix	LT630001.1
<i>Enterococcus faecalis</i> phage vB_EfaP_IME195	NC_028693
<i>Enterococcus faecalis</i> phage vB_Efae230P-4	NC_025467
<i>Enterococcus faecium</i> phage vB_EfaP_IME199	KT945995.1

Table S4: Features of Idefix ORFs and predicted functions of their products

ORF	Start-End position Translation initiation region*	Amino acids residues	Pi	Best BLASTp similarity with <i>Enterococcus</i> phages vB_EfaP_IME195 ^a or vB_Efae230P-4 ^b (E value ; % identity)	Predicted function (Eventual Comments)
IDF_00	197-355 AAATTTA <u>AGGAGG</u> AAACAACATG	52	4.94	^a YP_009191319.1 (9e-28 ; 98%)	Hypothetical protein
IDF_01	370-900 ATCTA <u>AGGAGG</u> AAAAATAAAAATG	176	4.65	^a YP_009191320.1 (4e-68 ; 67%)	Hypothetical protein
IDF_02	1041-1418 ACAGTAG <u>AGGAGA</u> ATTTATTATG	125	9.43	^b YP_009103979.1 (5e-57 ; 89%)	Single-stranded DNA binding protein
IDF_03	1486-1650 TATATC <u>AGGAGG</u> TAACAACAATG	54	4.06		Hypothetical protein
IDF_04	1663-1863 TGTTTTA <u>AGGAGG</u> AAACAAAATG	66	4.53		Hypothetical protein
IDF_05	1847-2269 CTACAGAG <u>AGGAGT</u> ATATTAATG	140	10.2	^a YP_009191326.1 (2e-96 ; 97%)	Hypothetical protein (putative terminal protein)
IDF_06	2262-3503 AGCTTATC <u>AGGACG</u> GTACAATG	413	6.22	^a YP_009191327.1 (0.0 ; 99%)	Encapsidation protein
IDF_07	3516-5864 TCAGTT <u>AGGAGT</u> TTTTACACATG	782	5.68	^b YP_009191328.1 (0.0 ; 98%)	DNA polymerase (B type, protein priming subfamily)
IDF_08	5924-6094 GGTTA <u>AGGAGG</u> AATTAATCATG	56	5.04	^a YP_009191329.1 (1e-32 ; 100%)	Hypothetical protein
IDF_09	6094-6303 GTA <u>ACGGAG</u> GTAGAAGCATAATG	69	7.98	^a YP_009191330.1 (7e-23 ; 65%)	Hypothetical protein
IDF_10	6294-6467 TGTA <u>CGGAGT</u> ACCAATTCAATG	57	4.32	^b YP_009103971.1 (8e-30 ; 91%)	Hypothetical protein
IDF_11	6468-6701 AGAAATT <u>GGAGG</u> ATCTTTAAATG	77	4.73	^a YP_009191332.1 (1e-26 ; 71%)	Hypothetical protein
IDF_12	6698-7201 GAACTAT <u>CGGAGG</u> AATTAATAATG	167	9.92	^a YP_009191333.1 (2e-117 ; 97 %)	HNH homing endonuclease
IDF_13	7194-8612 AATAAGAA <u>AGGGT</u> GTTGCTAATG	472	4.28	^a YP_009191334.1 (0.0 ; 97%)	Endolysin (PlyCA like, putative peptide LysIA of a multimeric endolysin)
IDF_14	8695-9051 TGGTATTAT <u>AAGT</u> TCTTAAATG	118	9.25	^b YP_009103967.1 (2e-58 ; 83%)	Hypothetical protein
IDF_15	9843-9067 ATCCGA <u>AGGGAG</u> GTGAATAAATG	258	7.67	^a YP_009191336.1 (0 ; 96%)	Endolysin (N-acetylmuramoyl-L-alanine amidase AmdI)
IDF_16	10236-9844 AACAAA <u>AGGAG</u> AAACGTTTAAATG	130	5.81	^a YP_009191337.1 (5e-89 ; 99%)	Holin
IDF_17	11990-10236 GATAG <u>AGGGG</u> ATTTTCTAATATG	584	6.79	^a YP_009191338.1 (0.0 ; 93%)	Tail protein
IDF_18	12777-11992 ACTTACT <u>AGGAGG</u> AGATACAATG	261	4.90	^a YP_009191339.1 (0.0 ; 95%)	Hypothetical protein
IDF_19	14354-12789 CATGGTAA <u>AGGAG</u> AGAAAAATATG	521	5.27	^a YP_009191340.1 (0.0 ; 98%)	Putative virion protein (calcineurin-like phosphoesterase motif)
IDF_20	15032-14367 GTT <u>AGGTG</u> GTGAACAACAAAATG	221	4.56	^a YP_009191341.1 (7e-126 ; 98%)	Lower collar protein
IDF_21	15999-14965 TATAACA <u>AGGAGG</u> TATTTAAATG	344	4.70	^a YP_009191342.1 (0.0 ; 98 %)	Head-tail connector
IDF_22	17229-16015 CCT <u>AGGAGG</u> GAGAAGAATAATATG	404	5.02	^a YP_009191343.1 (0.0 ; 96%)	Major head protein
IDF_22.1	17401-17231 AATA <u>AGGAG</u> AGTGCTAAGAAAATG	56	4.54	^b YP_009103958.1 (2e-31 ; 96%)	Hypothetical protein
IDF_23	17855-17496 CTTGACTATTTACTATATTATG	119	3.27	^b YP_009103957.1 (3e-49 ; 90%)	Hypothetical protein

*The putative RBS sequences are underlined and the start codons are indicated in bold

Table S5. Spontaneous mutations in *epaX* (*ef2170*) or 2169 in *pp6⁻* and resistance to Idefix infection

Clone	ORF Position	Mutation type	Reference	Mutation	Amino acids change	Resistance to Idefix
						% adsorption (sd)
VE18306 B7	<i>epaX</i> 2079401	IS		IS256 Opposite direction	Frameshift	+ 9.14 (7.3)
VE18306 B10	<i>epaX</i> 2079381	IS		IS256 Opposite direction	Frameshift	+ 7.04 (3.0)
VE18306 C6	<i>epaX</i> 2079493	IS		IS256 Same direction	Frameshift	+ 11.1 (8.3)
VE18306 C7	<i>epaX</i> 2079432	IS		IS256 Same direction	Frameshift	+ 14.2 (4.9)
VE18306 E1	<i>epaX</i> 2079518	IS		IS256 Same direction	Frameshift	+ 7.27 (4.0)
VE18306 G12	<i>epaX</i> 2079402	IS		IS256 Opposite direction	Frameshift	+ 8.29 (6.2)
VE18306 F2	<i>ef2169</i> 2077970-2078002	D	TGACTTCAGTTATCAGT AATCCAGAAATTAATA		11 amino acids deleted	+ 36.17 (6.6)
VE18306 F12	<i>ef2169</i> 2077970-2078002	D	TGACTTCAGTTATCAGT AATCCAGAAATTAATA		11 amino acids deleted	+ 45.4 (5.6)
	2078627-2078637	SNPs	CATAACTAAAT	ACATAACTAAA	5 amino acids substituted	
VE18306 F3	<i>ef2169</i> 2077970-2078002	D	TGACTTCAGTTATCAGT AATCCAGAAATTAATA		11 amino acids deleted	+ 28.3 (3.9)
	2078898	SNP	A	C	Tyr/Stop	

IS: Insertion Sequence, D: Deletion, SNP: Single Nucleotide Polymorphism