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Supplementary Materials for

A phage tail–like bacteriocin suppresses competitors in metapopulations of pathogenic bacteria

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The PDF file includes:

Figs. S1 to S12
Tables S1 to S5

Other Supplementary Material for this manuscript includes the following:

MDAR Reproducibility Checklist

Figure S1. The two modern and three historic collection sites in Southwestern Germany.

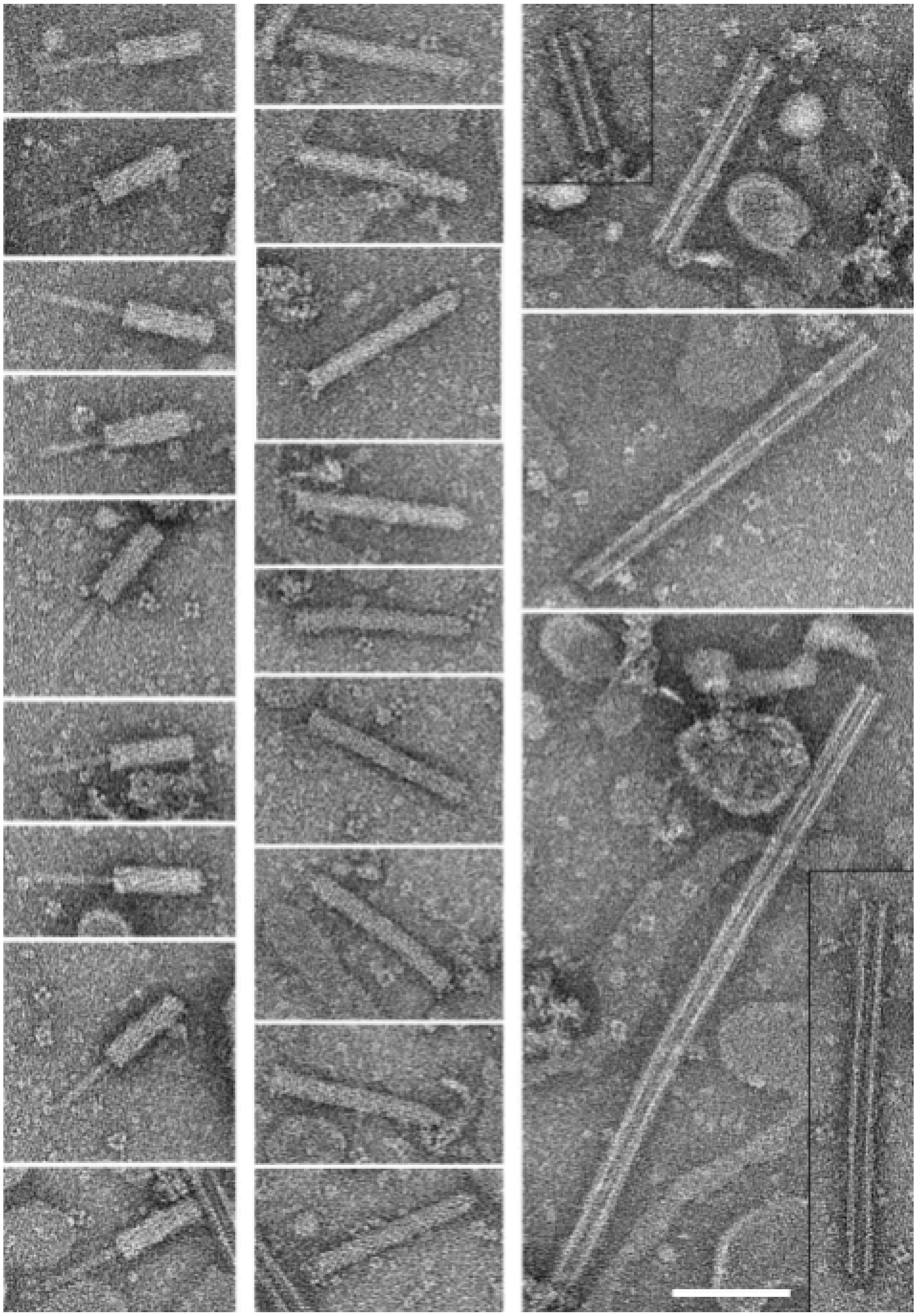

Figure S2. TEM images of p25.A12 contracted tailocins (left column), uncontracted tailocins (center column), and phage-like hollow tubes (right column). In the central column, the baseplate end of the tailocin faces left. The hollow tubes had highly variable lengths, average 404 (±153) nm (n = 14). Scale bar, 100 nm.

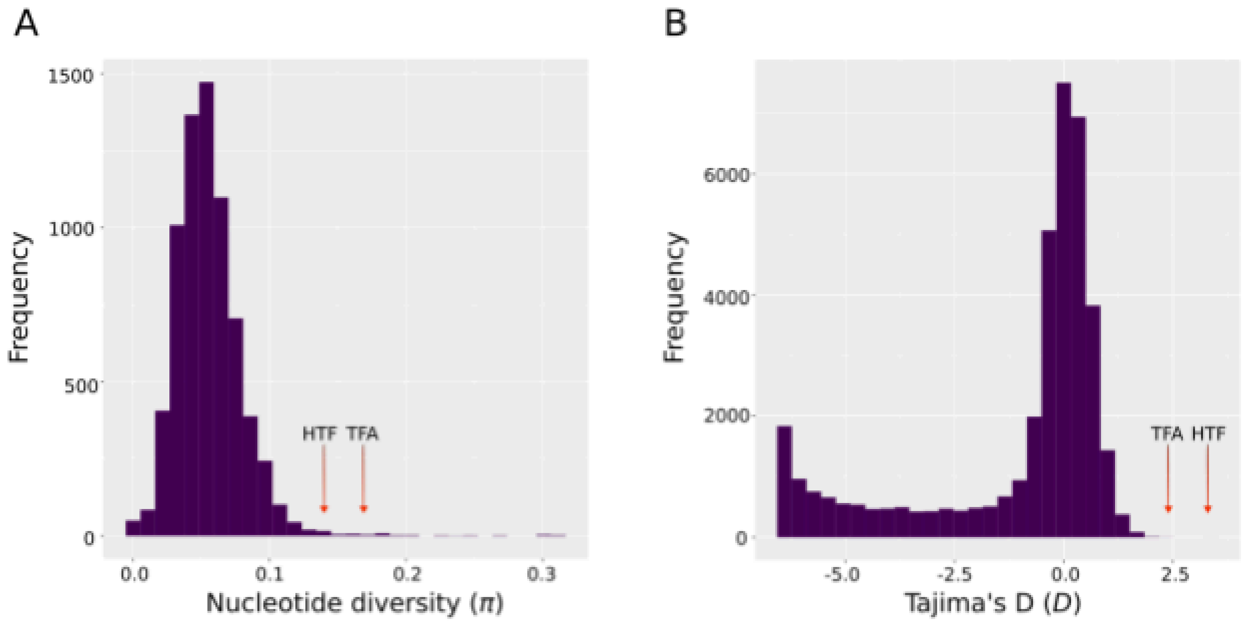

Figure S3 The tail fiber assembly gene and hypothetical tail fiber are evolving quicker than the rest of the bacterial genome. The distribution of **A.** nucleotide diversity (π) and **B.** Tajima's D (D) for all core genes in the 1,399 ATUE5 genomes. The TFA and HTF genes (red arrows) have significantly higher π and D compared to the rest of the core genes.

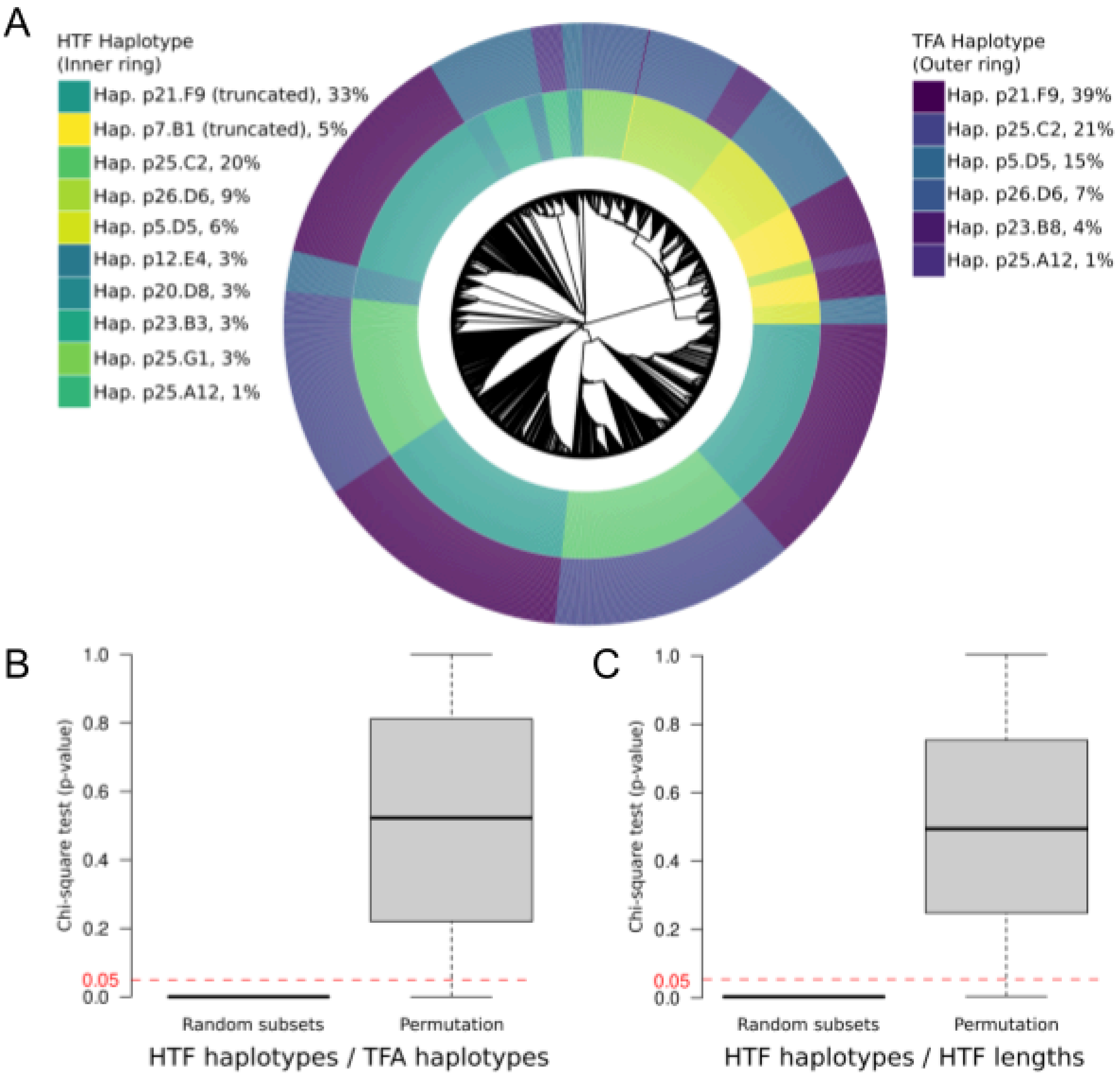

Figure S4. Associations between Tail Fiber Assembly (TFA) and Hypothetical Tail Fiber (HTF) haplotypes are significant. A. Maximum likelihood concatenated core genome phylogeny of 1,399 pathogenic pseudomonads that co-occur in the *Arabidopsis thaliana* phyllosphere with the HTF and TFA haplotypes plotted in the inner and outer rings. B-C. Each box plot represents a distribution of 1000 Chi-square test p-values drawn from random draws of pairs between TFA and HTF haplotypes and data sets for which HTF values were randomly permuted. Associations were tested between the two protein haplotypes (A) and between HTF haplotypes and TFA gene lengths (B).

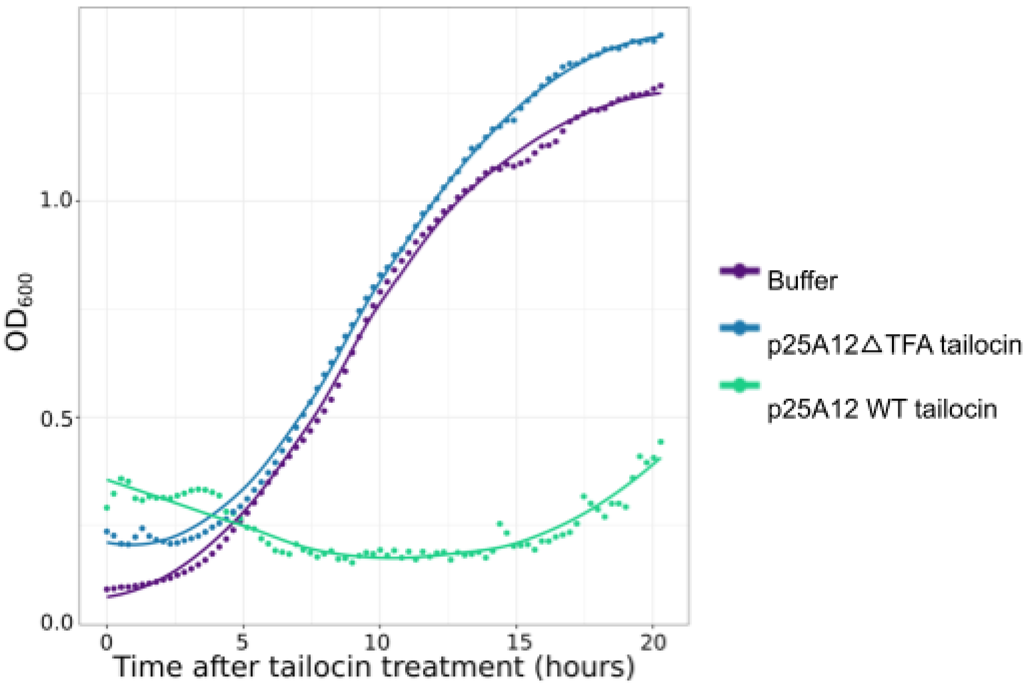

Figure S5. The tail fiber assembly gene was successfully knocked out and the knockout strain loses killing ability. A known sensitive strain to the tailocin (p25.C2) was grown and the OD_{600} was measured for 20 hours. Treatments included p25.A12 WT purified tailocin, p25.A12 mutant purified tailocin, and a buffer control. Each treatment was done in two independent replicates. Each dot represents the average OD_{600} with a trendline for each treatment.

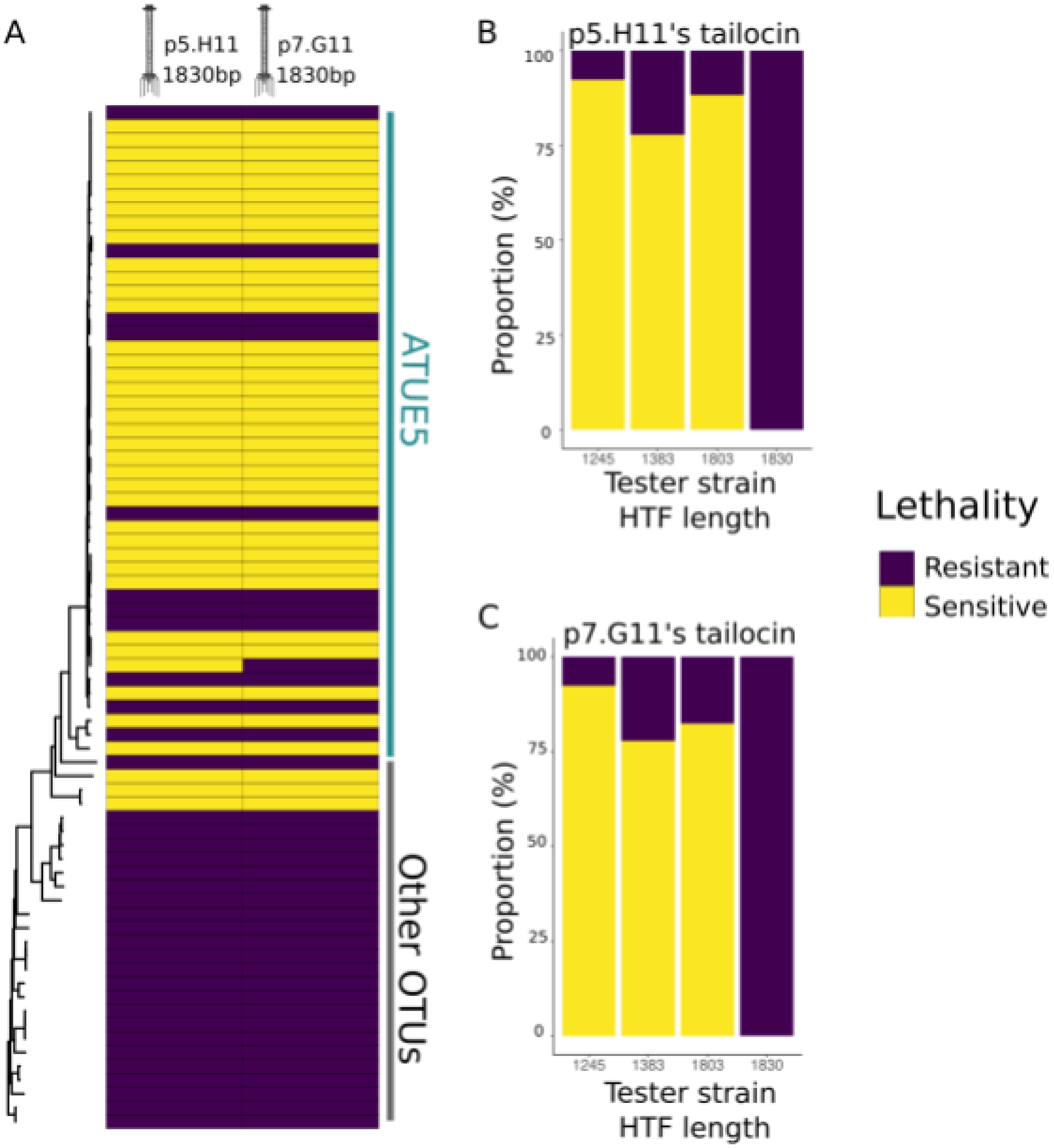

Figure S6. A. Tailocins are preferentially used for intra-lineage killing. Soft agar cultures of the *Pseudomonas* strains (rows) were challenged with viral particles extracted from cultures of two strains, p5.H11 and p7.G11 (from the 1830bp hypothetical tail fiber length haplotype, columns), in 3 technical replicates and 1 biological replicate. The phylogeny includes 83 *Pseudomonas* representative strains and are displayed according to their phylogenetic placement. Vertical lines indicate strains that belong to ATUE5 (colored in green) and other OTUs (colored in black). **B-C**. The proportion of tester strains sensitive or resistant to p5.H11's tailocin (B) and p7.G11's tailocin (C). Lethality significantly correlated with the tester strain's hypothetical tail fiber length haplotype (Fisher's Exact test, $p = 10^{-4}$ (B), $p = 10^{-3}$ (C).

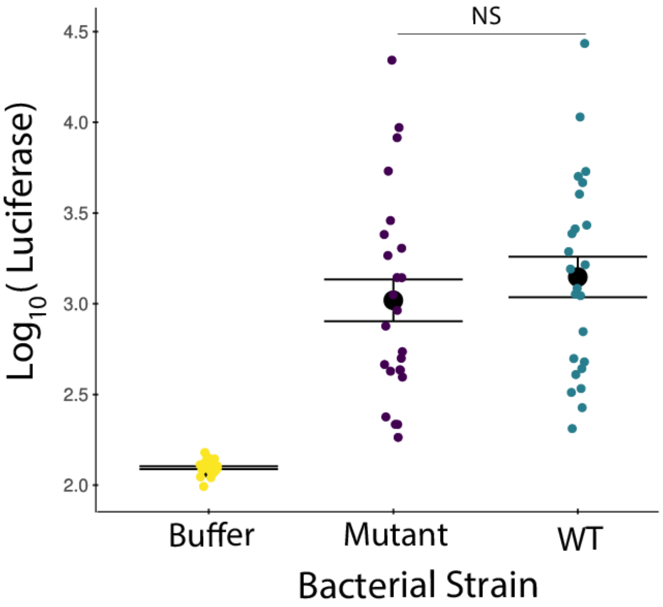

Figure S7. Growth of mutant and wild type p25.A12 *in planta*. Plants were infected in 24 technical replicates of a buffer control, mutant p25.A12, or wild type p25.A12 and luciferase was measured. ANOVA test, p-value 0.9.

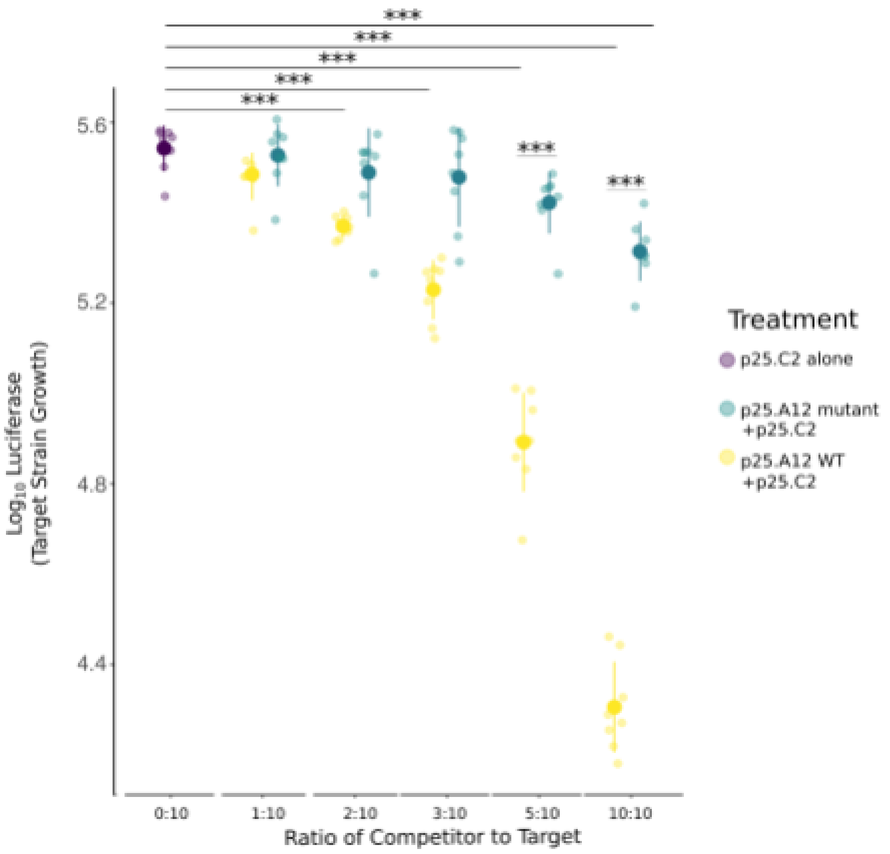

Figure S8. *In vitro* coinfections of p25.A12 (competitor) and p25.C2 (target, known to be sensitive to p25.A12). p25.A12 mutant competed with p25.C2 as well as p25.C2 grown alone were used as controls. Different ratios of competitor and constant amounts of target strain were used. p25.C2 grown alone as the control. The three highest values for each treatment was used and the average luciferase value was calculated for each condition. ANOVA test, P-values are shown as $p < 0.001$ ***, $p < 0.01$ **, or $p < 0.05$ *.

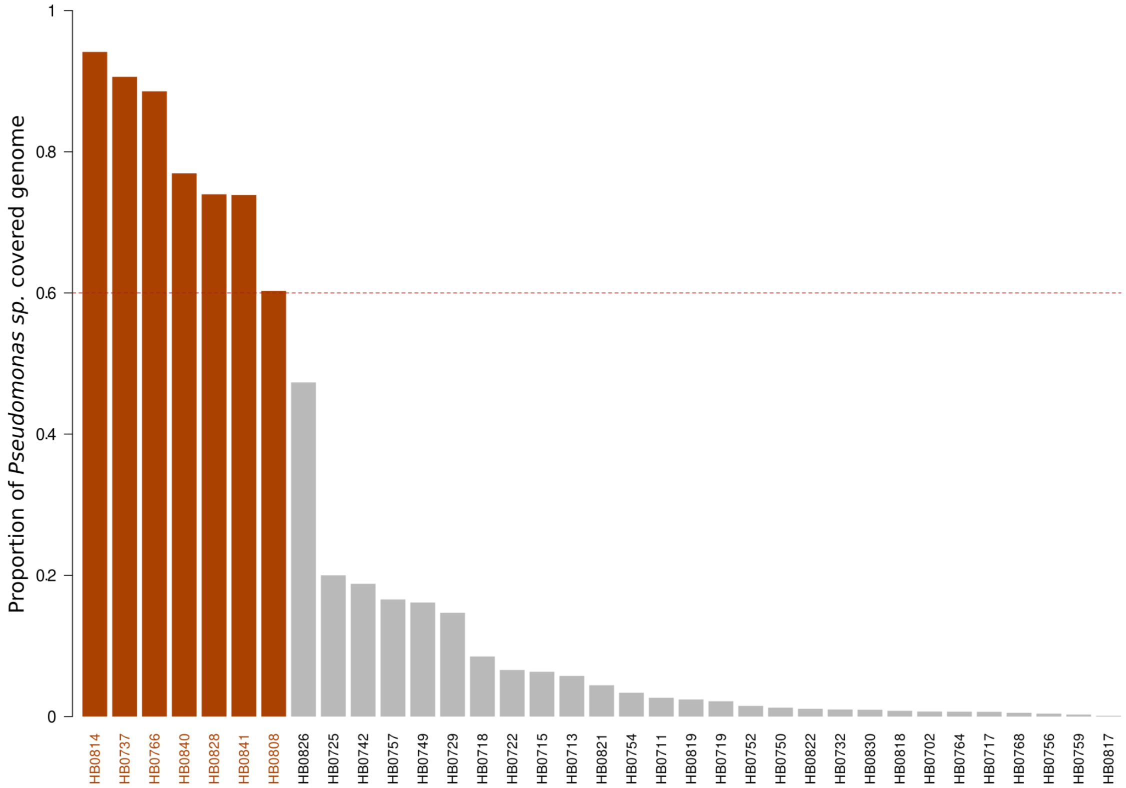

Figure S9. Seven historical samples cover at least 60% of the *Pseudomonas* genome. The height of the bars represents the proportion of the *Pseudomonas* sp. reference genome covered with at least one read after Mapping Quality filter value of 30. The horizontal dotted line depicts a threshold of 0.6.

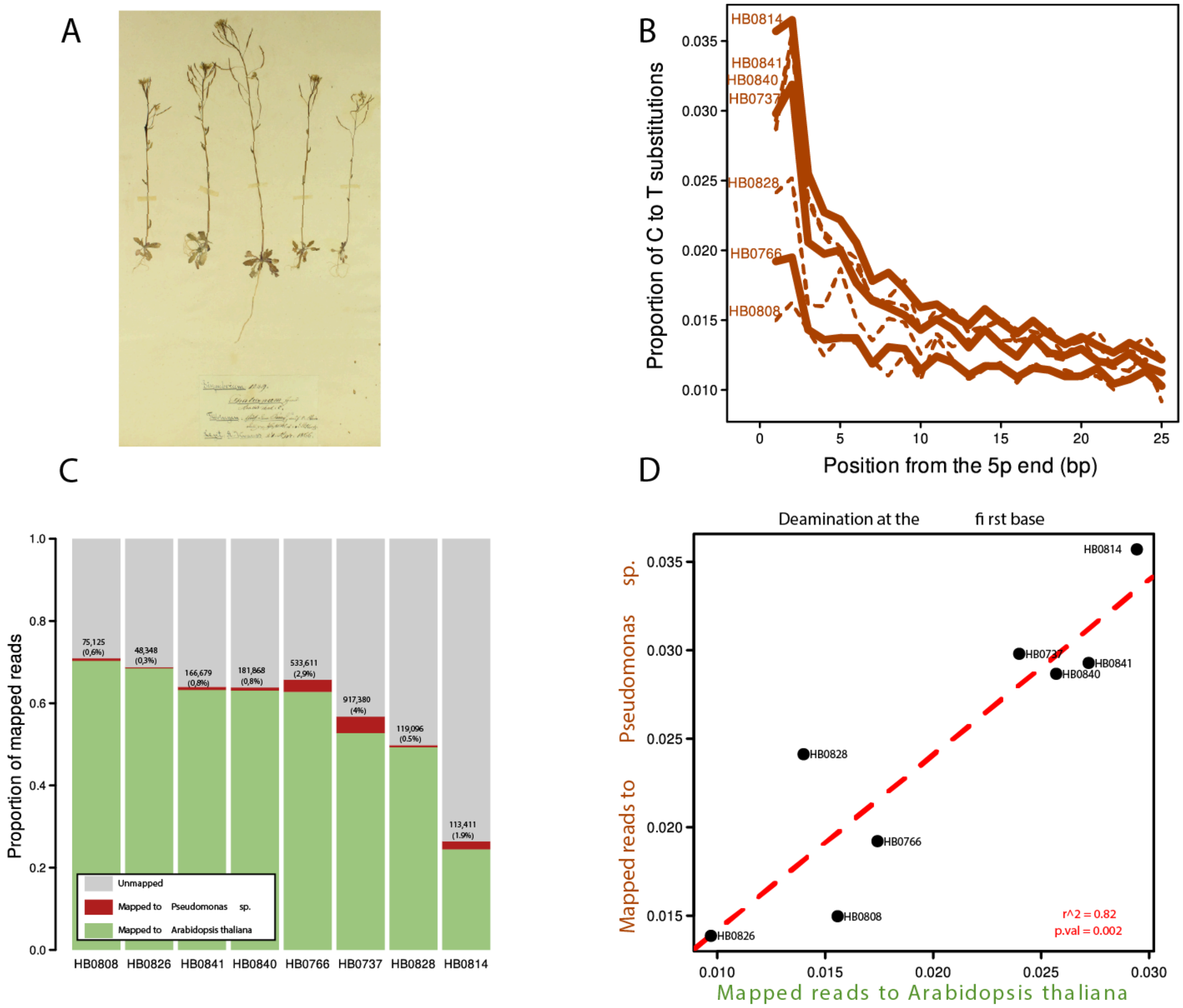

Figure S10. Herbarium-derived reads assigned to the host plant *Arabidopsis thaliana* and to *Pseudomonas* sp. show degradation patterns typical of ancient DNA. A. The photo shows a representative *A. thaliana* herbarium specimen (sample HB0729) sampled in the Tübingen herbarium and is part of the collection dataset previously reported in (*67*). **B**. Cytosine-to-Thymine (C-to-T) substitutions at the 5' end of reads mapped to the *Pseudomonas* viridiflava reference genome. Only samples that covered more than 60% of the *P. viridiflava* genome were included in the analysis. The thick lines indicate samples included in the phylogenetic analyses. **C.** The bars indicate the proportion of reads assigned to *A. thaliana* or to *Pseudomonas* sp. **D.** The scatter plot displays the relationship between the proportion of C-to-T substitutions at the first base of the 5' end between reads assigned to A. thaliana or to *Pseudomonas* sp. The red dotted line represents a linear model.

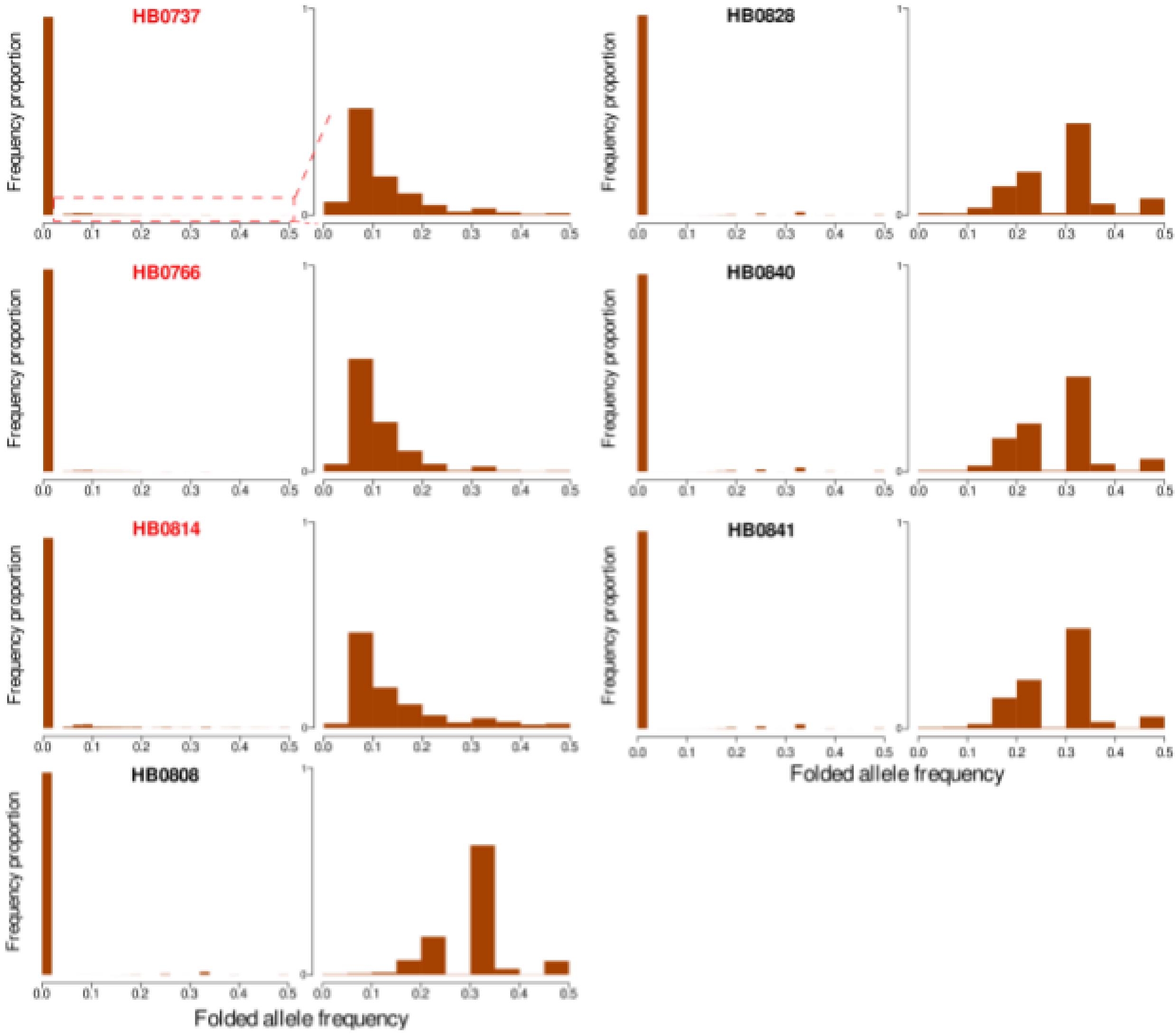

Figure S11. Historical *Arabidopsis thaliana* herbarium specimens are dominated by a single *Pseudomonas* sp. strain. The plots show the folded homozygosity support for each position along the *Pseudomonas viridiflava* genome with a coverage greater than 1x. A value of zero indicates that all reads support the same nucleotide (total homozygosity), where a value of 0.5 indicates maximum heterozygosity with only half of the reads supporting a particular nucleotide. Each row shows the folded homozygosity support for each sample. The left column shows all bins, whereas the bar indicating total homozygosity was removed in the right column.

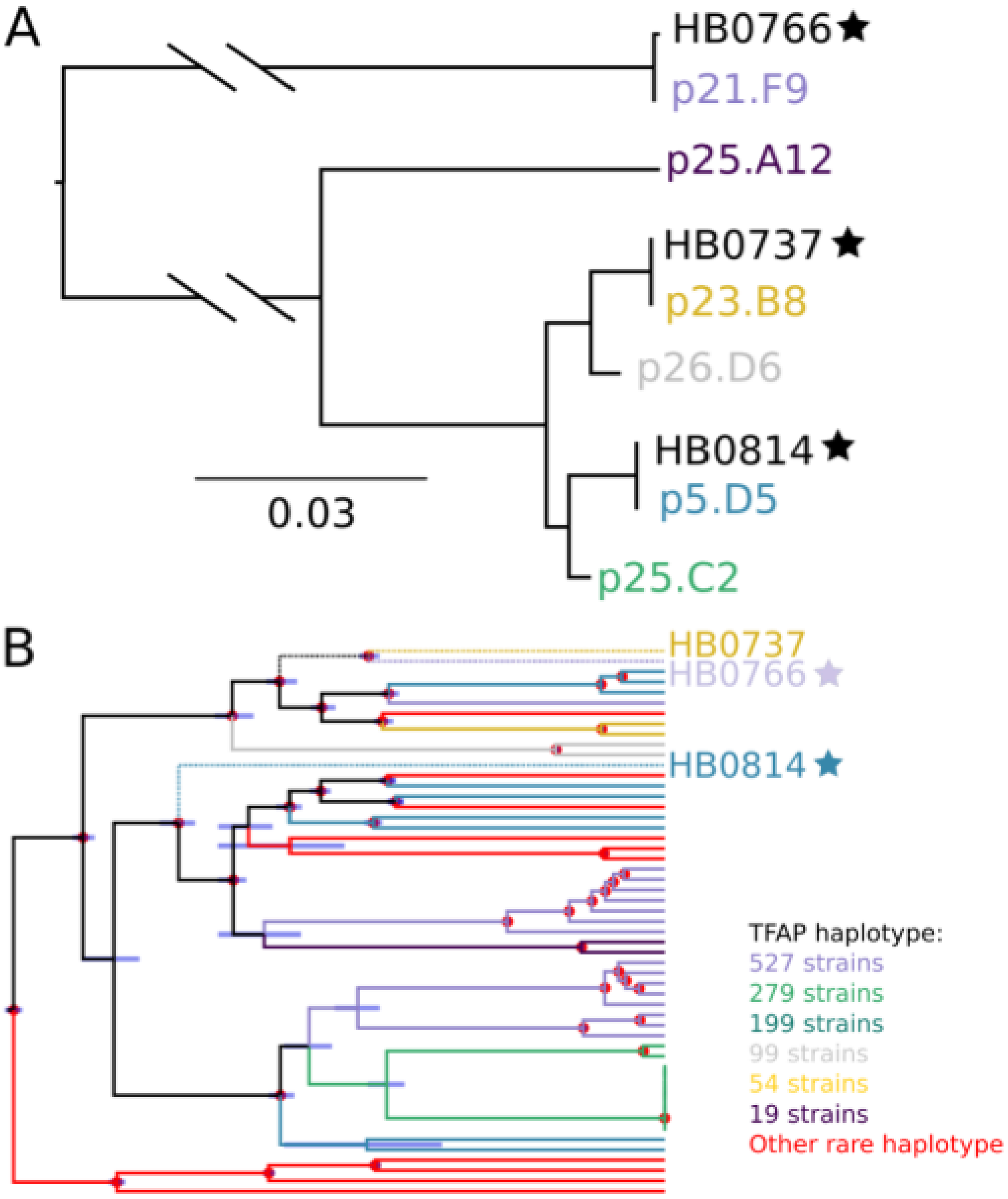

Figure S12. A. Neighbor-Joining tree of the tail fiber assembly gene translated sequence. Historical samples (HB) are placed in the context of the most common haplotypes. **B.** Bayesian tip-date calibrated phylogeny representing the evolutionary relationship between historical and modern *Pseudomonas* sp. strains. The tips and branches are colored based on the tail fiber assembly gene haplotype. Historical samples are shown with stars. The node bars represent the 95% Highest Posterior Density Intervals of the estimated time and the nodes marked with red dots represent those with posterior probability of 1.

Table S1. Untargeted data dependent LC/MS/MS Proteomics data for p25.A12 WT and p25.A12△TFA tailocin partially purified extracts.

Sample	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00762	Phage tail sheath protein	53.2	498	77	27	27	109		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00762	MAISFNNIPSDVRVPLFYAEMDNSAANSASAGMR	1xMet-loss [N-Term]	1	1	3515.66821	1172.56287	3	1.66	4.67
	JNHLCIDM_00762	RLIVAQVNDDVTGPEIGSLVLVPSVALAK		2	1	2973.6983	991.90564	3	1.37	4.62
	JNHLCIDM_00762	NDQHITIQAVEMAAPQPVWLQAAALAAR		3	0	3013.56763	1005.19531	3	1.24	4.39
	JNHLCIDM_00762	NAYGQADNSYLDSETMHQSAFIIR		12	0	2731.24167	911.08704	3	1.79	3.99
	JNHLCIDM_00762	ADPTGEVWCLPLLNTEGAK	1xCarbamidomethyl [C9]	7	0	2071.01647	1036.01257	2	0.68	3.74
	JNHLCIDM_00762	VQATVVNGATAAQAANALSVK		5	0	1984.08218	992.54694	2	2.23	3.45
	JNHLCIDM_00762	YGIATAYYEGGYVR		7	0	1582.75363	791.88129	2	1.05	3.38
	JNHLCIDM_00762	LIVAQVNDDVTGPEIGSLVLVPSVALAK		3	0	2817.59719	939.87231	3	1.85	3.12
	JNHLCIDM_00762	NLGGQGSMLAAMYETWR		3	0	1884.87311	942.94202	2	1.93	3.04

	JNHLCIDM_00762	SVVDAGVLTLSCK	1xCarbamidomethyl [C12]	4	0	1348.71408	674.86176	2	1.6	3.04
	JNHLCIDM_00762	LEEEGHVENAEVFAQHLIVER		5	0	2448.21538	816.74542	3	2.59	2.91
	JNHLCIDM_00762	TAVFISADASRPTQSGTMPGLDPAPASQR		6	0	2929.44724	977.15558	3	1.69	2.81
	JNHLCIDM_00762	FGAGQPIITPSTIR		4	0	1457.81109	729.40985	2	0.92	2.73
	JNHLCIDM_00762	RGTVGTLVAAGQLR		9	1	1398.81757	699.91278	2	0.51	2.46
	JNHLCIDM_00762	FTLTERESLLR		2	1	1364.75324	682.88141	2	1.69	2.33
	JNHLCIDM_00762	GELIAQYAR		1	0	1020.54727	510.77844	2	2.29	2.27
	JNHLCIDM_00762	INATPDLPVR		2	0	1095.61568	548.31262	2	2.08	2.06
	JNHLCIDM_00762	GTVGTLVAAGQLR		2	0	1242.71646	621.86285	2	1.59	1.98
	JNHLCIDM_00762	RLQGVITSK		2	1	1001.6102	501.30865	2	-0.17	1.82
	JNHLCIDM_00762	VFALLNQFR		10	0	1107.63094	554.31921	2	0.19	1.8
	JNHLCIDM_00762	MAISFNNIPSDVR	1xMet-loss [N-Term]	3	0	1332.69064	666.84991	2	1.44	1.79
	JNHLCIDM_00762	FGAGQPIITPSTIRGELIAQYAR		3	1	2459.34052	820.45215	3	0.56	1.34
	JNHLCIDM_00762	VPLFYAEMDNSAANSASAGMR		2	0	2201.99541	734.67078	3	1.07	1.27
	JNHLCIDM_00762	NDQHITIQAVEMAAPQPVWLQAAALAAR	1xOxidation [M12]	1	0	3029.56255	1010.52716	3	1.45	1.24
	JNHLCIDM_00762	QLYGHVYSAK		3	0	1165.60003	583.30603	2	4.08	1.23
	JNHLCIDM_00762	VNVMFPPDYINGLR		4	0	1634.83592	817.92236	2	0.93	1.14

	JNHLCIDM_00762	AGATVTVAGAATETGLLNLYVGGVR		2	0	2361.27725	787.76465	3	0.91	1.11
	JNHLCIDM_00762	DGNDPSRVNVMFPPDYINGLR		1	1	2376.1401	792.72034	3	2.67	0.86
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00761	Phage tail tube protein	12.5	115	68	7	7	24		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00761			6	0	1987.01647	994.01355	2	1.69	4.37
	JNHLCIDM_00761	TYVLSGAYLVEEPTSK		5	0	1756.90036	878.95569	2	2.13	3.02
	JNHLCIDM_00761			1	1	1967.87297	984.44226	2	2.17	2.89
	JNHLCIDM_00761	AVHTPDMPLK		2	0	1108.58194	554.79468	2	0.12	2.53
	JNHLCIDM_00761	VDGTQLTISGGGEAPLMNVK	1xOxidation [M17]	1	0	2003.01138	668.34283	3	1.28	2.33
	JNHLCIDM_00761	ADDGTIDLR		2	0	975.47416	488.24094	2	0.44	2.27
	JNHLCIDM_00761	RDTVVPGYFKEVDK		1	2	1652.86425	551.62689	3	1.14	1.7
	JNHLCIDM_00761	RDTVVPGYFK		1	1	1181.63133	394.54852	3	-0.27	1.48
	JNHLCIDM_00761	AVHTPDMPLK	1xOxidation [M7]	5	0	1124.57686	375.53104	3	1.51	1.4
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00046	hypothetical protein JNHLCIDM_00046	48.5	460	52	17	17	28		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr

	JNHLCIDM_00046	AAVDSTDLGNLWYSR		2	0	1667.80237	834.4057	2	1.05	2.76
	JNHLCIDM_00046	IAALGLSEVGSYAFAR		5	0	1624.86933	812.93933	2	1.26	2.68
	JNHLCIDM_00046			1	0	2318.09487	773.37079	3	1.27	2.58
	JNHLCIDM_00046			1	0	1893.93009	947.46997	2	1.36	2.45
	JNHLCIDM_00046			1	1	2050.03121	684.01672	3	2.15	2.26
	JNHLCIDM_00046	SVPGVGLVSGK		1	0	999.58332	500.29584	2	1.08	2.21
	JNHLCIDM_00046			3	0	1953.05524	977.0329	2	1.68	2.18
	JNHLCIDM_00046			2	0	2632.37294	878.13116	3	2.28	2.12
	JNHLCIDM_00046	ANRGTTLEAYGITNAYTK		1	1	1943.98213	648.66626	3	1.08	1.79
	JNHLCIDM_00046	AILGLAASDYKK		2	1	1249.71506	625.36182	2	1.03	1.78
	JNHLCIDM_00046	NFNPDDKANR		1	1	1190.55487	397.52283	3	-0.8	1.77
	JNHLCIDM_00046			3	0	2505.17799	1253.09766	2	4.01	1.26
	JNHLCIDM_00046			1	0	2116.95388	706.32239	3	-0.6	1.25
	JNHLCIDM_00046	GTTLEAYGITNAYTK		1	0	1602.80098	801.90369	2	-0.55	1.25
	JNHLCIDM_00046	ESDNGVVYLQSR		1	0	1430.65465	715.83258	2	2.26	1.22
	JNHLCIDM_00046	SGVAAGNYSR		1	0	981.47483	491.24109	2	0.07	1.17
	JNHLCIDM_00046	IGWSGSSLK		1	0	934.49926	467.75381	2	1.17	1.04
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	estA	estA	69.5	643	26	10	10	19		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr

	estA			4	0	2142.02506	1071.51807	2	1.77	3.45
	estA			4	1	2774.37037	925.46375	3	2.27	3.23
	estA	LADSAQVLQQAGAR		1	0	1427.76012	714.38489	2	1.67	2.75
	estA			2	0	2427.17866	1214.09607	2	2.56	2.17
	estA	FNEQLVSR		1	0	992.51597	496.76288	2	2.53	1.97
	estA			3	0	2833.44254	945.15436	3	2.11	1.93
	estA			1	0	3368.6692	1123.56396	3	2.42	1.93
	estA	STALTFSDQTR		1	0	1226.60116	613.80511	2	1.46	1.36
	estA	LFFNDR		1	0	811.40971	406.20889	2	0.98	1.03
	estA	GYNLTIGTSYR		1	0	1244.62698	622.81763	2	0.8	0.88
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00765	hypothetical protein JNHLCIDM_00765	12.2	112	15	1	1	1		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00765	VESAATVASEAAHTASR		1	0	1657.814	553.27771	3	2.76	1.17
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00764	hypothetical protein JNHLCIDM_00764	21.3	196	7	1	1	1		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00764	RGQEASEQLHAIR		1	1	1494.77716	498.93112	3	1.1	1.13
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00048	Chitinase class I	20	181	5	1	1	1		

	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00048	YDTGQLALR		1	0	1036.54218	518.77545	2	1.39	1.97
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00049	Bacteriophage lysis protein	18.3	169	4	1	1	1		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00049	LATADLR		1	0	759.43593	380.22162	2	0.04	1.1
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00039	hypothetical protein JNHLCIDM_00039	22.3	208	4	1	1	1		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00039	LNEFAEER		1	0	1007.47925	504.24396	2	1.38	1.05
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	JNHLCIDM_00044	Baseplate J-like protein	36.9	346	3	1	1	1		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00044	TQVDLAGDALR		1	0	1158.61133	579.80981	2	0.88	1.49
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12 WT	trpE	trpE	54.5	493	3	1	1	1		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	trpE	AILPAGTLSGAPK		1	0	1195.7045	598.35718	2	2.16	1.4
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		

p25.A12 WT	JNHLCIDM_00040	Mu-like prophage FluMu DNA circularization protein	51.9	499	2	1	1	1		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00040	AVMATVDAVR		1	0	1032.55064	516.77795	2	-1.95	0.64
	Accession	Description	MW [kDa]	# AAs	Coverage [%]	# Peptides	# Unique Peptides	# PSMs		
p25.A12△TFA	JNHLCIDM_00762	Phage tail sheath protein	53.2	498	56	22	22	81		
	Master Protein Accessions	Sequence	Modifications	# PSMs	# Missed Cleavages	Theo. MH+ [Da]	Observed m/z [Da]	Charge	λM [ppm]	XCorr
	JNHLCIDM_00762	ADPTGEVWCLPLLNTEGAK	1xCarbamidomethyl [C9]	2	0	2071.01647	1036.01428	2	2.33	4.17
	JNHLCIDM_00762	SVVDAGVLTLSCK	1xCarbamidomethyl [C12]	5	0	1348.71408	674.86176	2	1.6	2.57
	JNHLCIDM_00762	FGAGQPIITPSTIR		5	0	1457.81109	729.41052	2	1.84	2.4

Table S2: Relative mole percentage of monosaccharide residues detected by GC/MS.

	Mole %			
Residue/Strain	**p21.F1**	**p21.F9**	**p25.A12**	**p25.C2**
Rha	**63.4**	**11.1**	**9.6**	**72.3**
Man	**0.1**	**0.2**	**0.1**	**0.1**

Gal	**0.0**	**0.0**	**0.1**	**0.0**
Glc	**21.7**	**56.5**	**75.4**	**14.5**
Hep	**0.2**	**Tr.**	**0.0**	**0.0**
Kdo	**3.0**	**11.4**	**1.2**	**0.3**
FucNAc	**5.8**	**0.0**	**0.0**	**5.1**
QuiNAc	**1.1**	**0.0**	**0.6**	**0.8**
ManNAc	**0.3**	**1.9**	**0.9**	**0.6**
GalNAc	**0.3**	**1.1**	**0.7**	**0.3**
GlcNAc	**4.2**	**17.6**	**11.6**	**6.0**
% CHO	**60.6%**	**22.5%**	**31%**	**52%**

Tr.- trace

Table S3: Historic and modern sample metadata.

* Type: (H)istoric ; (M)odern

ACCESSION_ID	ENA_ID	TYPE*	COLLECTION_YEAR	COUNTRY	LOCATION	LAT	LON	Pseudomonas_Genome_Coverage_Prop.	Pseudomonas_Genome_Average_Depth_MQ30	Used_for_authentication_analyses	
HB0702	SRR21814520	H	1905	DEU	Mellendorf_near_Buttlar	52.5470675	9.7299326	0.00701769	0.00629547	NO	NO
HB0711	SRR21814519	H	1840	DEU	NA	NA	NA	0.0266046	0.0199101	NO	NO
HB0713	SRR21814508	H	1826	DEU	Esslingen	48.7427584	9.3071685	0.057587	0.0515586	NO	NO
HB0715	SRR21814497	H	1828	DEU	Esslingen	48.7427584	9.3071685	0.0634669	0.0581094	NO	NO

HB0717	SRR21814491	H	1826	DEU	Alpirsbach	48.3456021	8.4033079	0.00664118	0.00581024	NO	NO
HB0718	SRR21814490	H	1890	DEU	Tuebingen	48.5236164	9.0535531	0.0850372	0.0709387	NO	NO
HB0719	SRR21814489	H	1890	DEU	Tuebingen	48.5236164	9.0535531	0.0216538	0.0139758	NO	NO
HB0722	SRR21814488	H	1891	DEU	Altburg	48.6619431	8.6926366	0.0659517	0.0522472	NO	NO
HB0725	SRR21814487	H	1888	DEU	Tuebingen	48.5236164	9.0535531	0.199974	0.197268	NO	NO
HB0729	SRR21814486	H	1866	DEU	Tuebingen	48.5236164	9.0535531	0.146878	0.136151	NO	NO
HB0732	SRR21814518	H	1932	DEU	Rastatt	49.0532906	8.5245089	0.0100316	0.0070049	NO	NO
HB0737	SRR21814517	H	1937	DEU	Tuebingen	48.5236164	9.0535531	0.906105	8.75871	YES	YES
HB0742	SRR21814516	H	1953	DEU	Lichtenstein	48.41996913	9.26630222	0.187988	0.162055	NO	NO
HB0749	SRR21814515	H	1849	DEU	near_Hohenheim	48.7118	9.2113	0.161478	0.156122	NO	NO
HB0750	SRR21814514	H	1957	DEU	Boeblingen	48.684969	9.0113444	0.012546	0.010024	NO	NO
HB0752	SRR21814513	H	1952	DEU	Eberspiel_in_the_district_of_Calw	48.7415506	8.6842699	0.0150715	0.0118002	NO	NO
HB0754	SRR21814512	H	1951	DEU	District_of_Calw	48.7153344	8.7381796	0.033677	0.028471	NO	NO
HB0756	SRR21814511	H	1951	DEU	Wendlingen	50.8021737	7.7130698	0.00412007	0.00351381	NO	NO
HB0757	SRR21814510	H	1951	DEU	Wendlingen	50.8021737	7.7130698	0.165843	0.100545	NO	NO
HB0759	SRR21814509	H	1893	DEU	Wohlmuthausen	50.5727241	10.21353915	0.00280539	0.00201518	NO	NO
HB0764	SRR21814507	H	1905	DEU	Mengen	48.049796	9.3316096	0.00683053	0.00540983	NO	NO
HB0766	SRR21814506	H	1846	DEU	Monastery_forest_Hohenzollern	47.9724751	10.3005044	0.885642	7.63767	YES	YES
HB0768	SRR21814505	H	1894	DEU	Todtnau	47.8303378	7.9452378	0.00528544	0.00427861	NO	NO
HB0808	SRR21814504	H	1817	DEU	Stuttgart_Moehringen	48.7784485	9.1800132	0.60285	0.974844	YES	NO
HB0814	SRR21814503	H	1882	DEU	Heidelberg	49.4093582	8.694724	0.941364	8.13508	YES	YES
HB0817	SRR21814502	H	1934	DEU	Gundelwangen_near_Bonndorf	47.8426163	8.2864051	0.00113424	0.00213973	NO	NO
HB0818	SRR21814501	H	1928	DEU	Langenau_near_Ulm	48.7276553	8.0150864	0.00812181	0.00744537	NO	NO
HB0819	SRR21814500	H	1928	DEU	Langenau_near_Ulm	48.7276553	8.0150864	0.0241737	0.0120816	NO	NO
HB0821	SRR21814499	H	1874	DEU	Winnenden	48.8754571	9.3978478	0.0443252	0.0396616	NO	NO

HB0822	SRR21814498	H	1936	DEU	Hohentwiel	47.7617515	8.8348709	0.0109147	0.0104388	NO	NO
HB0826	SRR21814496	H	1948	DEU	Abtsgmuend	48.8936471	10.0027636	0.473287	0.665255	NO	NO
HB0828	SRR21814495	H	1890	DEU	Schramberg	48.225478	8.3852168	0.739741	1.6142	YES	NO
HB0830	SRR21814494	H	1862	DEU	near_Beimerstetten	48.4840643	9.9836241	0.00970948	0.00894266	NO	NO
HB0840	SRR21814493	H	1851	DEU	Sulz_am_Neckar	48.3617509	8.6314329	0.769394	1.61748	YES	NO
HB0841	SRR21814492	H	1875	DEU	Freudenstadt_in_the_Black_Forest	48.4637727	8.4111727	0.738722	1.44545	YES	NO
p12.A11	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.955211	43.2805	NA	YES
p12.A9	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.385298	2.68517	NA	NO
p12.E2	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.946364	124.361	NA	YES
p12.F2	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.941151	61.1632	NA	YES
p12.G7	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.942483	26.1727	NA	YES
p12.H7	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.703408	7.44366	NA	NO
p13.C1	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.919667	91.4711	NA	YES
p13.C7	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.915668	30.2041	NA	NO
p13.D10	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.917782	83.4618	NA	YES
p13.D5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.373545	3.2645	NA	NO
p13.F1	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.548638	9.89508	NA	NO
p13.F3	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.930129	180.182	NA	YES
p13.F5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.486993	7.68403	NA	NO
p20.B10	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.362051	6.0333	NA	NO
p20.D4	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.965996	26.275	NA	YES
p20.F10	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.951949	27.3486	NA	YES
p20.G9	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.951606	25.8164	NA	YES
p21.A8	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.951989	50.4423	NA	YES
p21.E3	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.912764	42.5976	NA	YES
p21.F1	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.921125	54.2176	NA	YES
p21.F9	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.951456	43.9553	NA	YES
p22.A8	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.910773	42.5346	NA	YES
p22.B5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.908795	31.8141	NA	YES
p22.C1	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.910697	44.4703	NA	YES
p22.D1	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.93509	9.86993	NA	YES

p22.D4	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.901368	6.48617	NA	YES
p23.A3	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.922147	90.3312	NA	YES
p23.A5	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.355851	5.69028	NA	NO
p23.B4	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.353754	10.3529	NA	NO
p23.B8	NA	M	2018	DEU	Pfrondorf2	48.561087	9.109294	0.915323	25.8754	NA	YES
p24.B5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.906998	24.3191	NA	YES
p24.H2	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.919194	36.961	NA	YES
p25.A12	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.904389	8.27369	NA	YES
p25.B2	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.99527	9.73957	NA	YES
p25.C11	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.936486	7.67613	NA	NO
p25.C2	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.999957	38.9734	NA	YES
p25.D2	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.997331	11.0366	NA	YES
p26.B7	NA	M	2018	DEU	Pfrondorf2	48.561087	9.109294	0.918811	18.5831	NA	YES
p26.C10	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.532614	7.27294	NA	NO
p26.D6	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.907977	10.1768	NA	YES
p26.E7	NA	M	2018	DEU	Pfrondorf2	48.561087	9.109294	0.915118	26.4647	NA	YES
p26.F6	NA	M	2018	DEU	Pfrondorf2	48.561087	9.109294	0.263831	1.09767	NA	NO
p27.C5	NA	M	2018	DEU	Pfrondorf2	48.561087	9.109294	0.33096	4.98267	NA	NO
p27.D6	NA	M	2018	DEU	Pfrondorf2	48.561087	9.109294	0.915116	62.9111	NA	YES
p27.F2	NA	M	2018	DEU	Pfrondorf2	48.561087	9.109294	0.908864	30.4347	NA	YES
p3.A3	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.999479	12.1995	NA	YES
p3.F12	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.999766	64.6266	NA	YES
p3.F8	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.910145	14.5013	NA	NO
p3.G11	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.405107	35.7165	NA	NO
p3.G9	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.936757	40.7657	NA	YES
p4.A6	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.999359	11.257	NA	YES
p4.A8	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.317566	8.57523	NA	NO
p4.C5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.281958	2.19208	NA	NO
p4.D11	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.329928	7.72875	NA	NO
p4.D2	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.903089	43.5342	NA	YES
p4.E5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.905147	9.07888	NA	YES
p4.E6	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.907851	26.4274	NA	YES
p4.H3	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.359271	11.1964	NA	NO

p5.A5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.569164	20.9644	NA	NO
p5.C1	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.940631	83.5842	NA	YES
p5.C3	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.911686	61.8474	NA	YES
p5.D5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.908126	47.1102	NA	YES
p5.F2	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.355109	10.8067	NA	NO
p5.H11	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.912223	24.6014	NA	YES
p6.A10	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.967914	86.1443	NA	YES
p6.B5	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.377432	8.17665	NA	NO
p6.B9	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.915641	40.9034	NA	NO
p6.D10	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.362251	6.29957	NA	NO
p6.E9	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.348102	5.45088	NA	NO
p6.F1	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.441901	14.1066	NA	NO
p6.G2	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.591204	22.8581	NA	NO
p6.G3	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.374359	12.0216	NA	NO
p7.F2	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.420044	6.6193	NA	NO
p7.G11	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.922476	63.4571	NA	YES
p8.B3	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.999766	72.1507	NA	YES
p8.B9	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.921803	46.3749	NA	YES
p8.C7	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.933317	51.4209	NA	YES
p8.D11	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.432979	9.92024	NA	NO
p8.E4	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.917628	39.6076	NA	YES
p8.G10	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.460138	12.253	NA	NO
p8.G2	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.450816	8.26752	NA	NO
p8.H7	NA	M	2018	DEU	Det-2	48.556255	9.135424	0.899835	67.821	NA	YES
p9.C4	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.946827	40.897	NA	NO
p9.H10	NA	M	2018	DEU	Eyach	48.446111	8.781611	0.469617	11.8808	NA	NO
BAV2572	NA	M	2013	USA	Virgina	NA	NA	NA	NA	NA	NO
CDRTc14	NA	M	2013	AUT	Steinberg-Dörfl	47.4N	16.47E	NA	NA	NA	NO
ICMP8820	NA	M	2010	NZL	NA	NA	NA	NA	NA	NA	NO
ICMP3272	NA	M	1975	NZL	Riverhead	NA	NA	NA	NA	NA	NO
CFBP1590	NA	M	1974	FRA	Vaucluse	NA	NA	NA	NA	NA	NO

DSM6694	NA	M	1930	CHE	NA	NA	NA	NA	NA	NA	NO
CH409132	NA	M	2013	USA	Boston, Massachusetts	42.22N	71.7W	NA	NA	NA	NO
LMCA8460	NA	M	2008	USA	Lake Michigan College	42.090114N	86.393408W	NA	NA	NA	NO
SV1779	NA	M	2017	USA	Scott Valley, California	NA	NA	NA	NA	NA	NO
T157	NA	M	2019	USA	Tulelake, California	NA	NA	NA	NA	NA	NO

Table S4: Key Resources Table

REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Deposited Data		
1524 *Pseudomonas* genomes	Karasov et al. 2018	ENA: PRJEB24450
Experimental Models: Organisms/Strains		
1524 Modern *Pseudomonas* strains	Karasov et al. 2018	NA
34 Historic strains		In Table S3 (Sample)
10 NCBI genomes	NCBI	In Table S3 (Sample)
p25.A12 △TFA p25.A12△TFA +TFA	This study	NA
Software and Algorithms		
VIBRANT v1.2.1	Kieft et al., 2020	https://github.com/AnantharamanLab/VIBRANT
Mash v2.2.2	Ondov et al., 2016	https://github.com/marbl/Ma

		sh
panX	Ding et al., 2018	https://github.com/neherlab/pan-genome-analysis
pankmer	Aylward et al., 2023	https://pypi.org/project/pankmer/

Product	Primers	Size (bp)
p25.A12 △TFA attB DNA construction	upstream forward, 5′ ggggacaagtttgtacaaaaaagcaggctCGCGCGCGTCATCAATTCTA 3’ and upstream reverse 5’ TTTCAAAGAACCAGTTAAGGGTATTCATgatcctctag 3’; downstream forward, 5’ tgcaagcttgCAGGAGTTCACATGCCTATCG 3’ and downstream reverse 5’ ggggaccactttgtacaagaaagctgggtGTGCGTGAGCTGGGCAAC 3’	458bp
Flanking arm screening amplification	F-5′ CGCGCGCGTCATCAATTCTA 3′ and R-5′ GTGCGTGAGCTGGGCAAC 3′	WT = 946bp KO = 400bp
p25.A12 TFA with RE overhangs	F-5’atgcaagcttATGCACACAGTATTAAGCGCTCGC 3’ and R-5’ACGAGCTGCCCAAGAC	568bp

	CTGAggatccactagt 3’	

Table S5. De-novo assembly contigs statistics from reads mapped to the Tailocin region.

MAETRIC\SAMPLE	**HB0737**	**HB0766**	**HB0814**
Total_n	10	9	14
Total_seq_bp	23769	21573	21574
Avg._seq_bp	2376.9	2397	1541
Median_seq_bp	2143	1665	1275.5
N_50_bp	2858	3405	1705
Min_seq_bp	583	494	415
Max_seq_bp	5543	7935	3337