

Supporting Information to: “Expanding the Actinomycetes Landscape for Phosphonate Natural Products through Genome Mining”

Alina Zimmermann^{1,2}, *Shu-Ning Xia*^{2,3}, *Julia Moschny*^{2,3}, *Juan Pablo Gomez-Escribano*¹,
Judith Boldt^{1,4}, *Ulrich Nübel*^{1,4,5,6}, *Imen Nouioui*¹, *Janina Krause*³, *Mattis Kreins Irle*¹,
*William W. Metcalf*⁷, *Chambers C. Hughes*^{2,3,8}, *Yvonne Mast*^{1,2,5,6*}

¹Leibniz Institute DSMZ -German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany

²German Center for Infection Research (DZIF), Partner Site Tübingen, 72076 Tübingen, Germany

³Department of Microbial Bioactive Compounds, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany

⁴German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, 38124 Braunschweig, Germany

⁵Braunschweig Integrated Centre of Systems Biology (BRICS), Rebenring 56, 38106 Braunschweig, Germany

⁶Technische Universität Braunschweig, Institut für Mikrobiologie, Spielmannstr. 7, 38106 Braunschweig, Germany

⁷Carl R. Woese Institute for Genomic Biology, 1206 West Gregory Drive, Urbana, IL 61801, United States of America

⁸Cluster of Excellence EXC 2124: Controlling Microbes to Fight Infection, University of Tübingen, 72076 Tübingen, Germany

Table of Contents

Table of Contents	2
Figure S1. Gene Cluster Families of identified and known P-BGCs	4
Figure S2. ³¹ P NMR spectra of concentrated supernatant from <i>Kibdelosporangium banguiense</i> DSM 46670 and <i>Saccharopolyspora spinosa</i> DSM 44228 in different media.....	5
Figure S3. ³¹ P NMR spectra of concentrated supernatant from <i>Streptomyces aureocirculatus</i> DSM 40386 and <i>Streptomyces iranensis</i> DSM 41954 in different media	6
Figure S4. ³¹ P NMR spectra of concentrated supernatant from <i>Streptomyces mutomycini</i> DSM 41691 and <i>Kitasatospora cheerisanensis</i> DSM 101999 in different media	7
Figure S5. ³¹ P NMR spectra of concentrated supernatant from <i>Streptomyces glauciniger</i> DSM 41867 and <i>Streptomyces seoulensis</i> DSM 41840 in different media	8
Figure S6. ³¹ P NMR spectra of concentrated supernatant from <i>Kitasatospora fiedleri</i> DSM 114396 and <i>Streptomyces</i> sp. Tü H45 in different media	9
Figure S7. Synteny analysis for defining the boundaries of the putative P-BGC.	10
Figure S8. <i>clinker</i> analysis for defining the boundaries of the putative P-BGC.	12
Figure S9. Visualization of the putative P-BGC and GC-Frame plot in Artemis.	18
Figure S10. ³¹ P NMR spectra of concentrated supernatants from heterologous expression strains.....	19
Figure S11. BLAST analysis of amino acid sequences from the <i>Kitasatospora fiedleri</i> P-BGC	20
Figure S12. Alignment of aldehyde dehydrogenase enzymes from P-BGCs.....	20
Figure S13. Overview of characterized enzyme functions in phosphonate biosynthetic pathways detected as predicted proteins encoded in <i>K. fiedleri</i> pBGC.....	22
Table S1. Identified potential phosphonate producer strains from the DSMZ and Tübingen collection and known producers of reduced phosphorus compounds included in the analysis	23
Table S2. Bioassay results in the test of putative phosphonate producers against <i>Escherichia coli</i> WM6242, <i>E. coli</i> K12 and <i>Kocuria rhizophila</i>	37
Table S3. Plasmids used in this study.....	38
Table S4. Strains used in this study	38
Table S5. Oligonucleotides used in this study	39

<i>Table S6.</i> Proposed phosphonate biosynthetic gene cluster from <i>Kitasatospora fiedleri</i> DSM 114396, based on the chromosome sequence with NCBI accession NZ_OX419519.1	41
---	----

Figure S1. Gene Cluster Families of identified and known P-BGCs

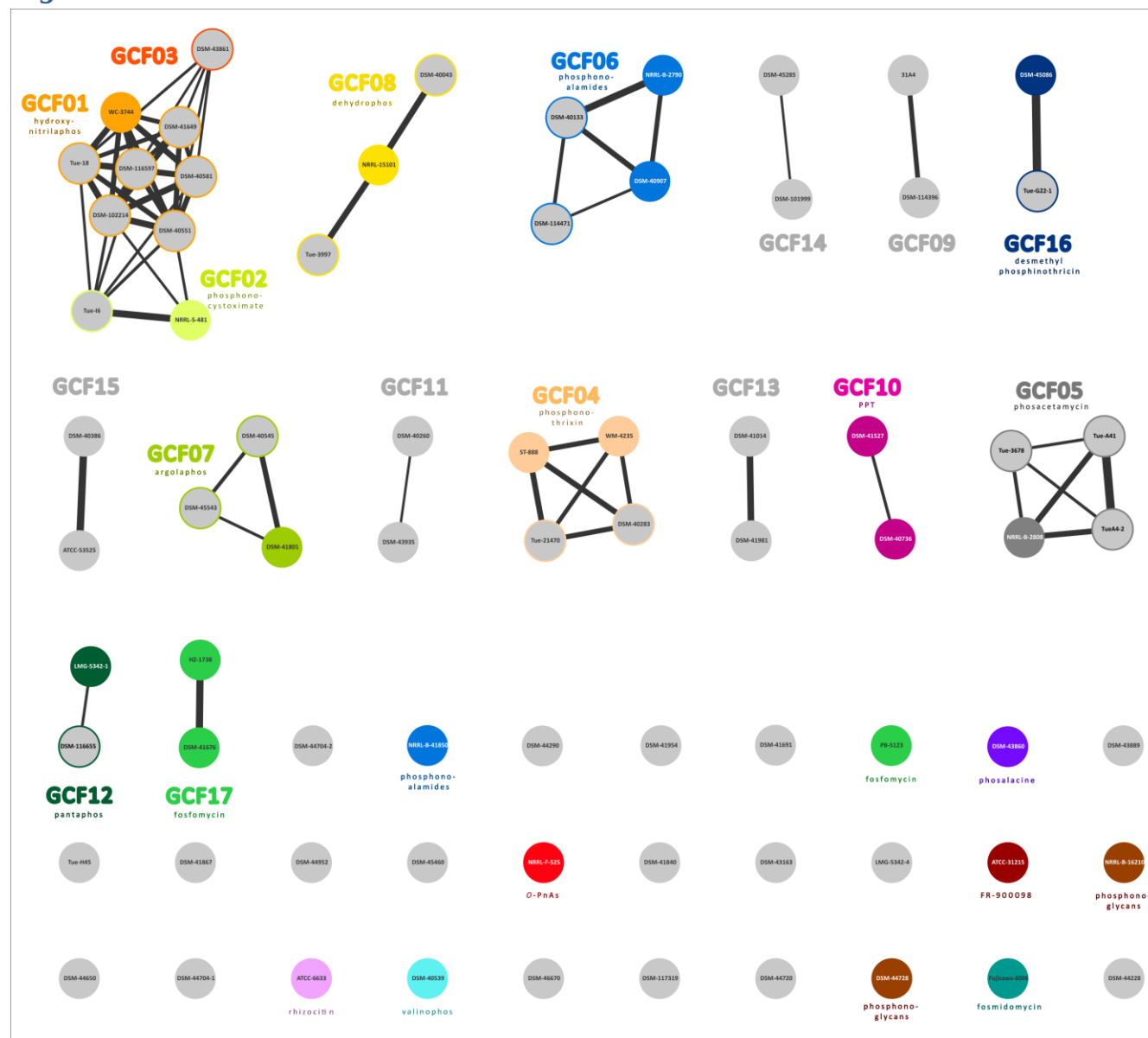
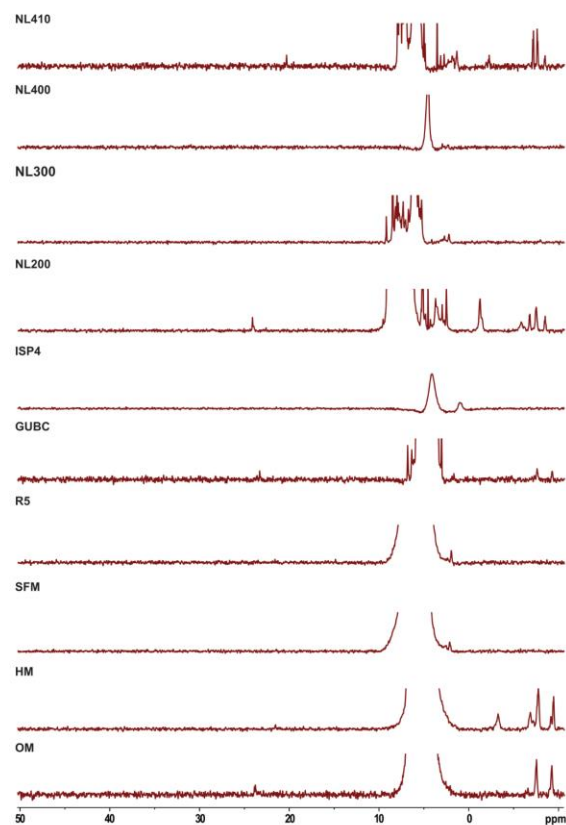


Figure S1. Gene cluster family (GCF) network as obtained from the BiG-SCAPE analysis and visualized with Cytoscape. Each node corresponds to a BGC. Colored spheres represent GCFs/singleton with sequences from previously identified phosphonate producers, light grey spheres are P-BGCs with unknown phosphonate product (for references see Table S1). Edges between two nodes represent a distance between the P-BGCs below a BiG-SCAPE cutoff threshold of 0.612 and distances are further represented by weighted lining of edges (lines get thicker with decreased distance).

Figure S2. ^{31}P NMR spectra of concentrated supernatant from *Kibdelosporangium banguiense* DSM 46670 and *Saccharopolyspora spinosa* DSM 44228 in different media

Baffled flasks containing OM medium, HM medium, SFM medium, R5 medium, GUBC medium, ISP4 medium, NL200 medium, NL300 medium, NL400 medium and NL410 medium were inoculated with starter culture. Cultures were incubated on a rotary shaker for 7 days. Culture supernatants were concentrated and analyzed by ^{31}P NMR spectroscopy. L-phosphinothricin HCl (10 mM in H_2O , δ_{P} 51.2 ppm) was used as a chemical shift reference. Signals with chemical shifts > 8 ppm are putative phosphonate-containing compounds.

***Kibdelosporangium banguiense* DSM46670**



***Saccharopolyspora spinosa* DSM44228**

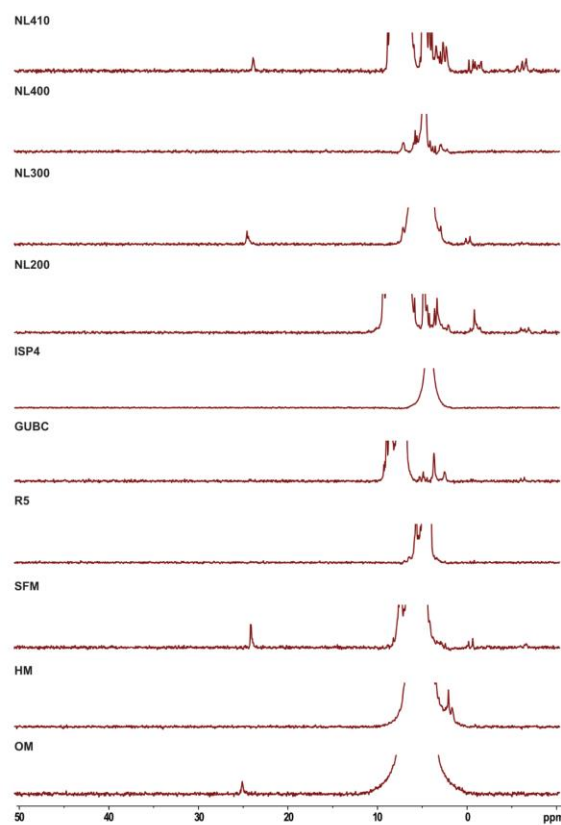
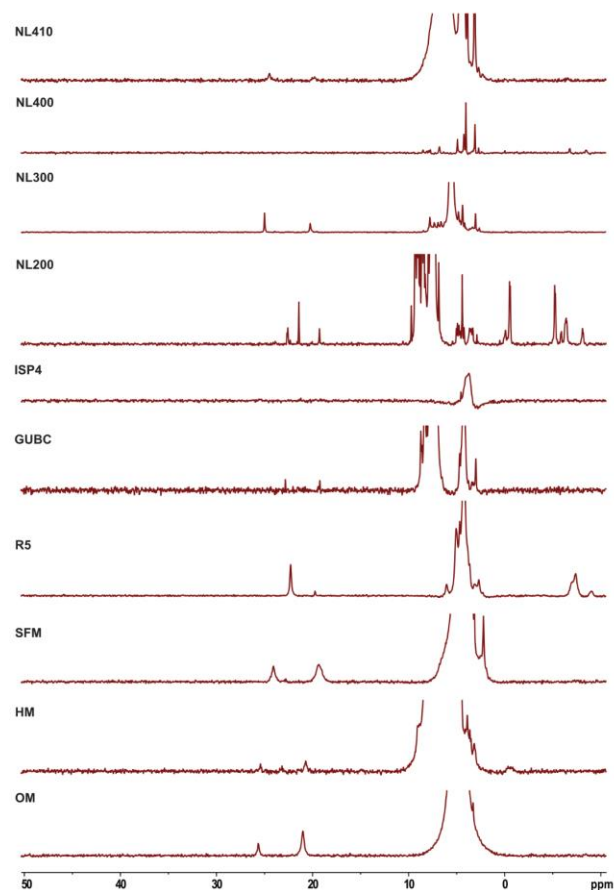


Figure S3. ^{31}P NMR spectra of concentrated supernatant from *Streptomyces aureocirculatus* DSM 40386 and *Streptomyces iranensis* DSM 41954 in different media

Baffled flasks containing OM medium, HM medium, SFM medium, R5 medium, GUBC medium, ISP4 medium, NL200 medium, NL300 medium, NL400 medium and NL410 medium were inoculated with starter culture. Cultures were incubated on a rotary shaker for 7 days. Culture supernatants were concentrated and analyzed by ^{31}P NMR spectroscopy. L-phosphinothricin HCl (10 mM in H_2O , δ_{P} 51.2 ppm) was used as a chemical shift reference. Signals with chemical shifts > 8 ppm are putative phosphonate-containing compounds.

***Streptomyces aureocirculatus* DSM40386**



***Streptomyces iranensis* DSM41954**

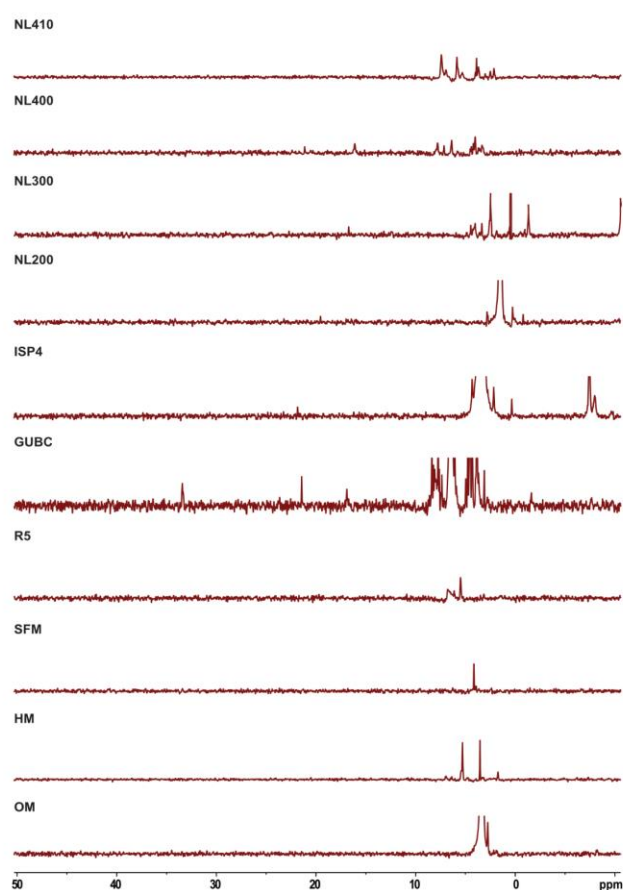
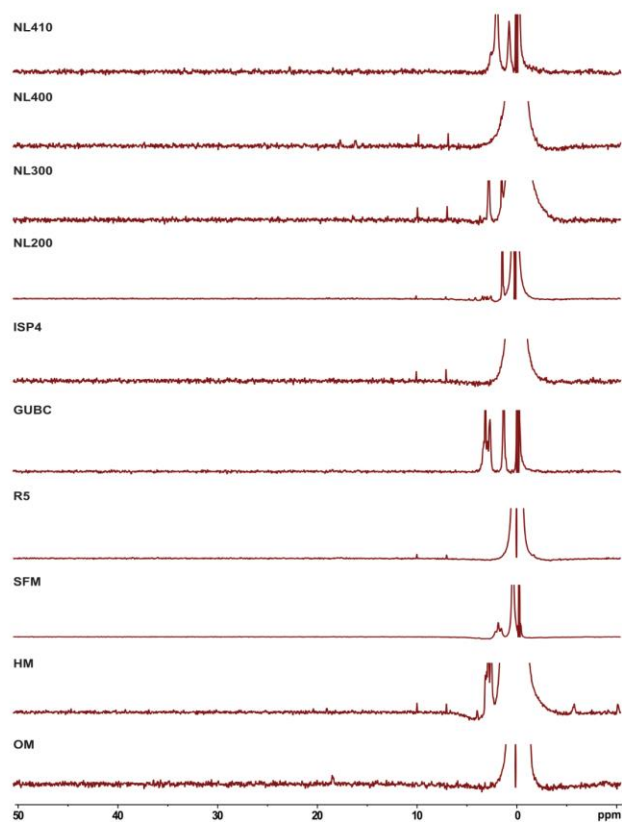


Figure S4. ^{31}P NMR spectra of concentrated supernatant from *Streptomyces mutomycini* DSM 41691 and *Kitasatospora cheerisanensis* DSM 101999 in different media

Baffled flasks containing OM medium, HM medium, SFM medium, R5 medium, GUBC medium, ISP4 medium, NL200 medium, NL300 medium, NL400 medium and NL410 medium were inoculated with starter culture. Cultures were incubated on a rotary shaker for 7 days. Culture supernatants were concentrated and analyzed by ^{31}P NMR spectroscopy. L-phosphinothricin HCl (10 mM in H_2O , δ_{P} 51.2 ppm) was used as a chemical shift reference. Signals with chemical shifts > 8 ppm are putative phosphonate-containing compounds.

***Streptomyces mutomycini* DSM41691**



***Kitasatospora cheerisanensis* DSM101999**

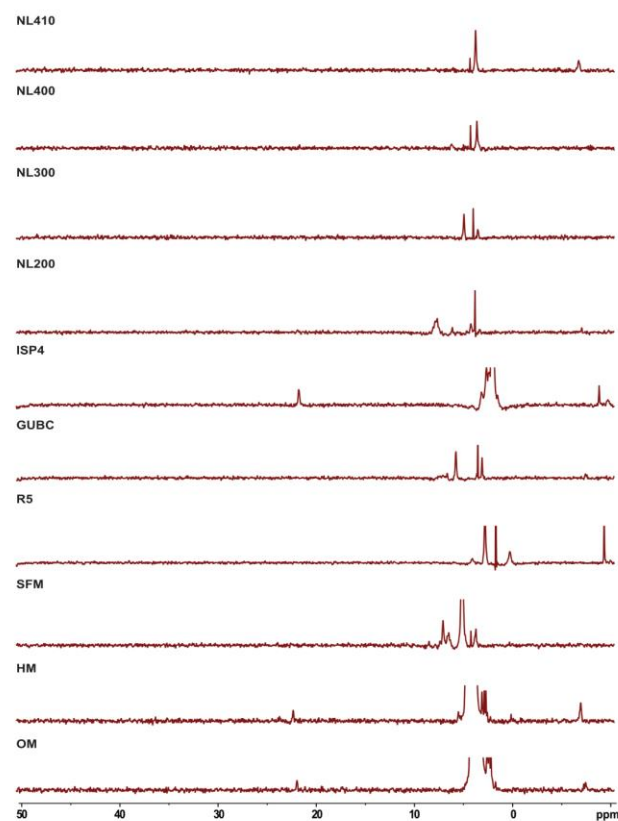
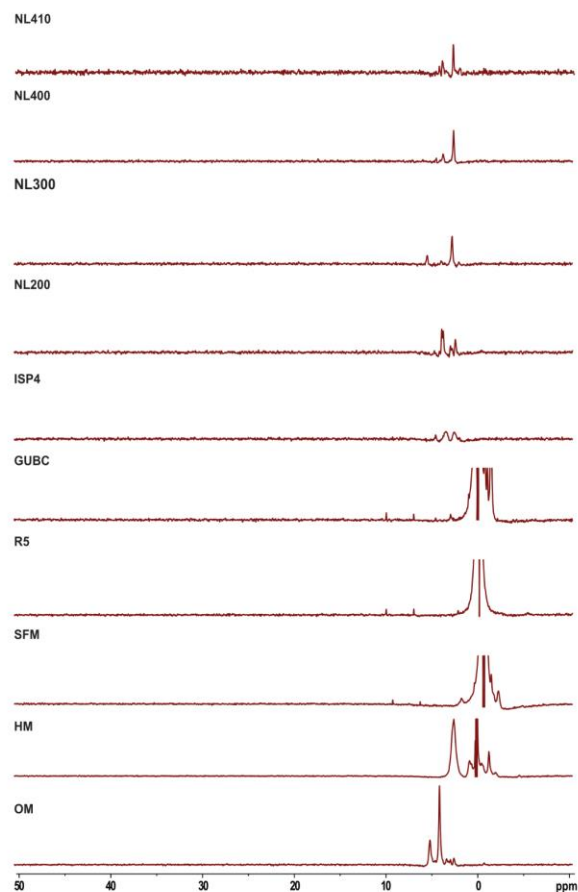


Figure S5. ^{31}P NMR spectra of concentrated supernatant from *Streptomyces glauciniger* DSM 41867 and *Streptomyces seoulensis* DSM 41840 in different media

Baffled flasks containing OM medium, HM medium, SFM medium, R5 medium, GUBC medium, ISP4 medium, NL200 medium, NL300 medium, NL400 medium and NL410 medium were inoculated with starter culture. Cultures were incubated on a rotary shaker for 7 days. Culture supernatants were concentrated and analyzed by ^{31}P NMR spectroscopy. L-phosphinothricin HCl (10 mM in H_2O , δ_{P} 51.2 ppm) was used as a chemical shift reference. Signals with chemical shifts > 8 ppm are putative phosphonate-containing compounds.

***Streptomyces glauciniger* DSM41867**



***Streptomyces seoulensis* DSM41840**

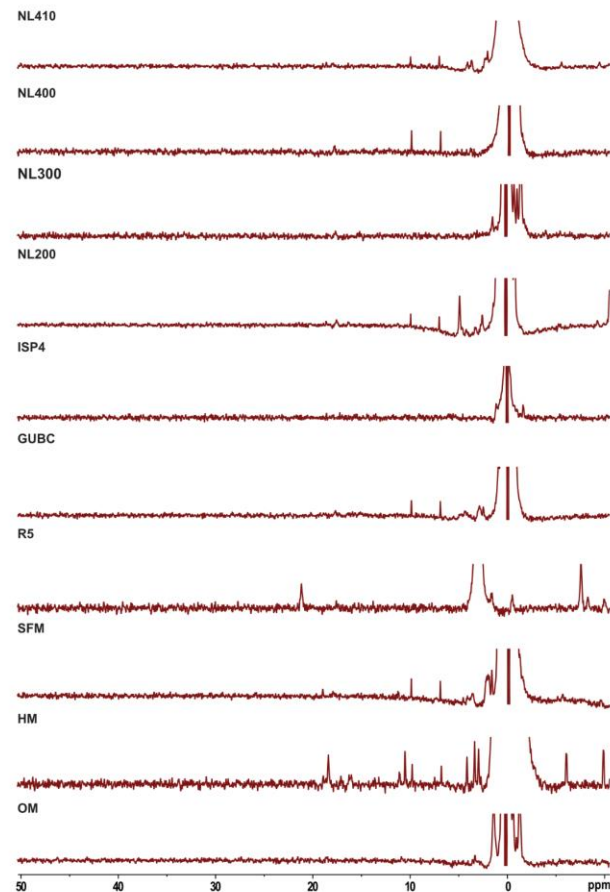
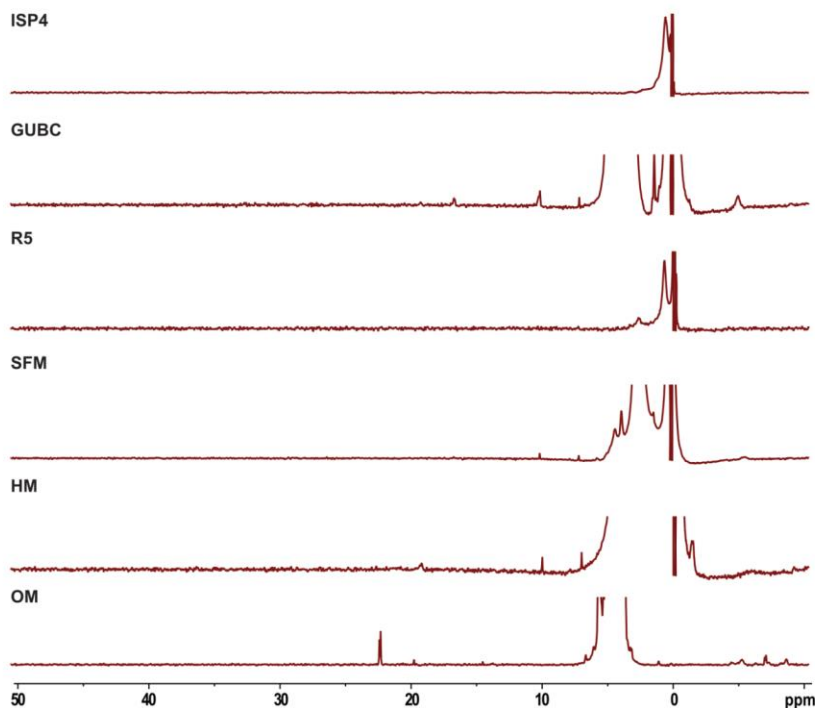


Figure S6. ^{31}P NMR spectra of concentrated supernatant from *Kitasatospora fiedleri* DSM 114396 and *Streptomyces* sp. Tü H45 in different media

Baffled flasks containing OM medium, HM medium, SFM medium, R5 medium, GUBC medium and ISP4 medium were inoculated with starter culture. Cultures were incubated on a rotary shaker for 7 days. Culture supernatants were concentrated and analyzed by ^{31}P NMR spectroscopy. L-phosphinothricin HCl (10 mM in H_2O , δ_{P} 51.2 ppm) was used as a chemical shift reference. Signals with chemical shifts > 8 ppm are putative phosphonate-containing compounds.

***Kitasatospora fiedleri* DSM114396**



***Streptomyces* sp. Tü H45**

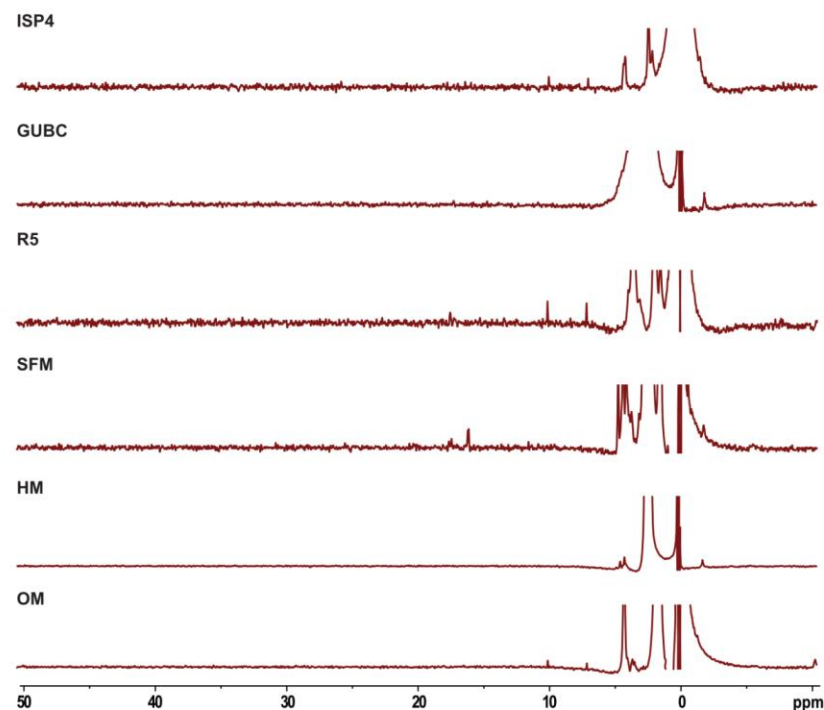
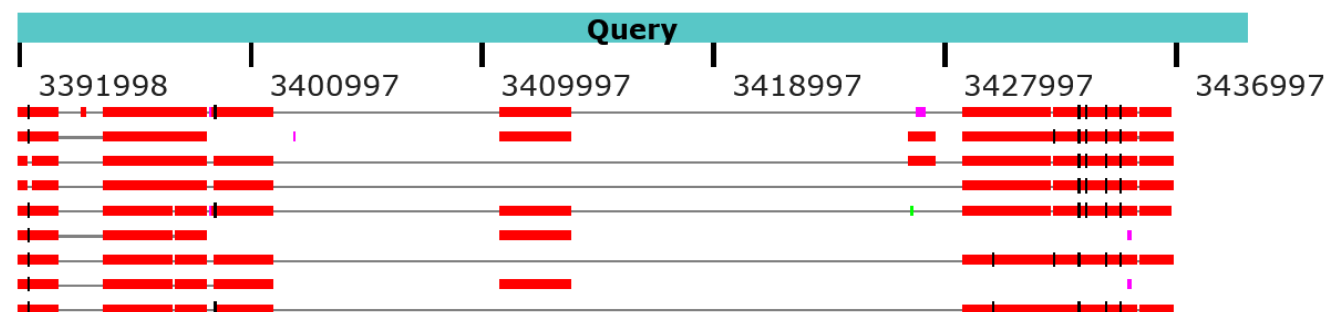


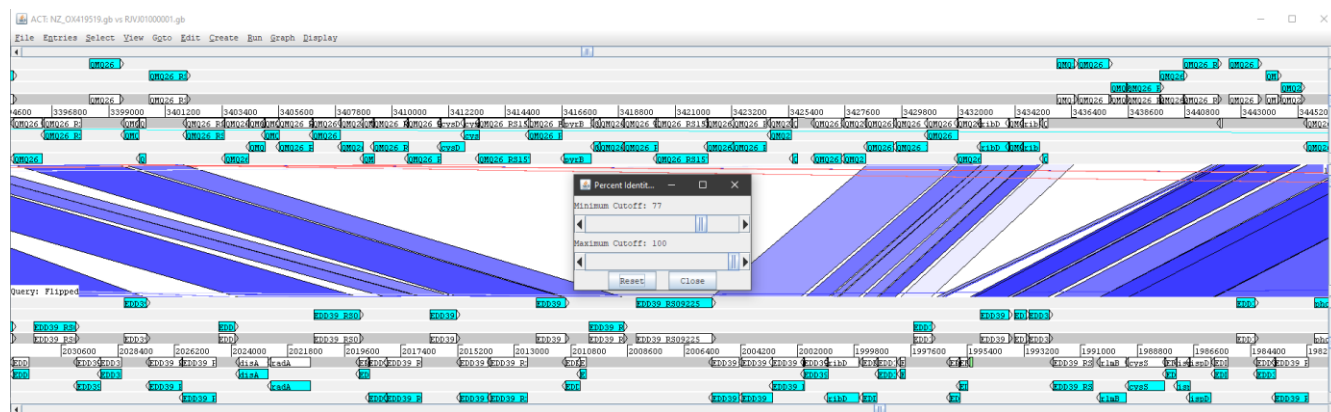
Figure S7. Synteny analysis for defining the boundaries of the putative P-BGC.

A. Similarity search with NCBI BLAST blastn program, using as query *Kitasatospora fiedleri* DSM 114396 chromosome sequence with accession NZ_OX419519, limited to the range 3391998-3439448, against the NCBI Database “Refseq prokaryote representative genomes (contains refseq assembly) Update date 2025/05/12” limited to “*Kitasatospora cineracea* (taxid:88074)”, the closest known species to DSM 114396 and for which several genome assemblies are available. The result shows the highly conserved regions (see also Fig. S8 **ABDG**) surrounding the putative phosphonate biosynthetic gene cluster, therefore helping to determine the boundaries of the gene cluster. The highly conserved region around position 3409997 in the image represents the segment 3410635 to 3413339 and contains the genes with locus_tag QMQ26_RS15735, QMQ26_RS15740 and QMQ26_RS15745, all of them homologs of the highly conserved genes with locus_tag QMQ26_RS09285 (*cysN*), QMQ26_RS09280 (*cysD*), and QMQ26_RS09275 (*cysC*), that span from position 1960006 to 1962829 of the chromosome of DSM 114396, and encode the highly conserved enzymes sulphate adenylyltransferase subunit 1 CysN [EC:2.7.7.4; KEEG K00956], sulphate adenylyltransferase subunit 2 CysD [EC:2.7.7.4; KEEG K00957], and adenylylsulphate kinase CysC [EC:2.7.1.25; KEEG K00860] from sulphur metabolism in bacteria (see also Fig. S3 and S5). The upstream end (right in Figure S3) is supported by a synteny analysis with the type strains of *Kitasatospora cineracea* and *Kitasatospora niigatensis*, the most closely related species to *K. fiedleri* lacking a P-BGC¹ (Figure S3). The downstream end (left in the figure) is well-supported also by highly conserved homologous genes beyond the aldehyde dehydrogenase, with the first gene (encoding a chloride channel protein) showing 69-97% identity to *Kitasatospora* strains in the NCBI non-redundant protein database. **B and C.** Artemis Comparison Tool (ACT) visualisation of the NCBI BLAST analysis of DSM 114396 chromosome (NZ_OX419519) against *K. cineracea* DSM 44780 contig NZ_RJVJ01000001 (**B**) or against *Kitasatospora niigatensis* DSM 44781 contig NZ_RKQG01000001 (**C**), using NCBI megablast with default options. Note that the display of the bottom sequence has been flipped in both B and C to match the orientation of the top one. **D.** ACT visualisation of the NCBI BLAST analysis of NZ_OX419519 against *Kitasatospora setae* KM-6054 (the type strain of the genus) chromosome (NC_016109.1) showing that the synteny restarts only with the gene with locus_tag QMQ26_RS15885 encoding a tRNA-Thr, as a result of being *K. setae* a more distant species.

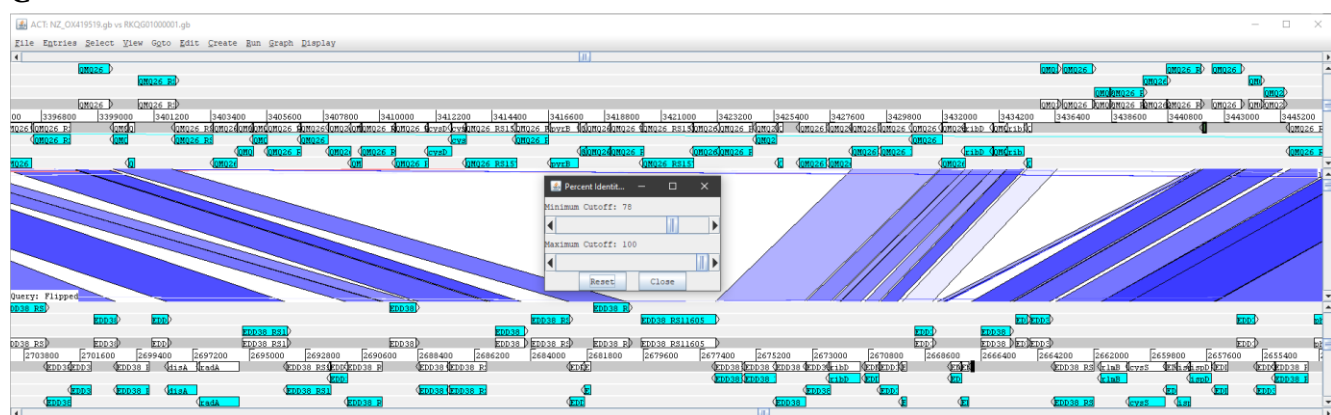
A



B



C



D

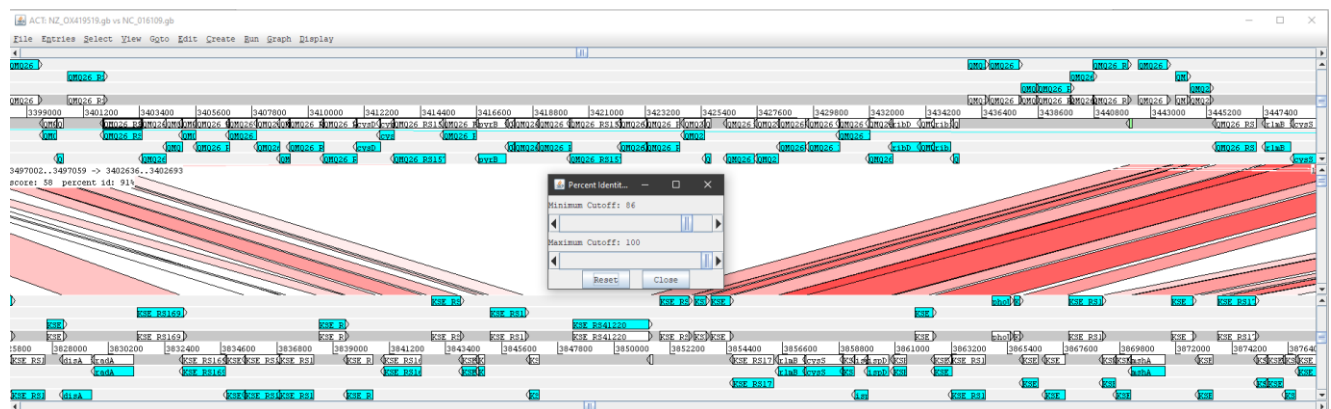
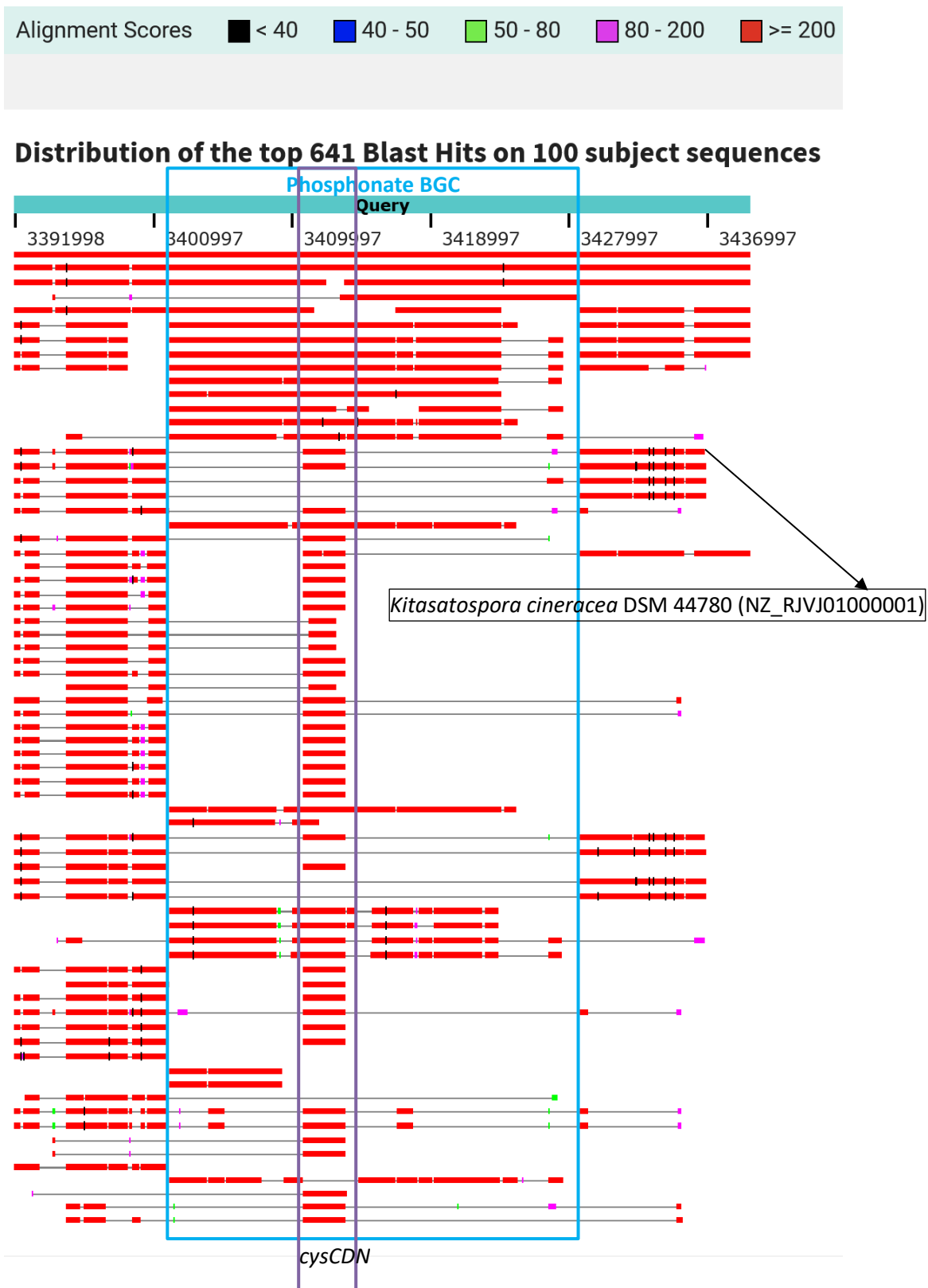


Figure S8. *clinker* analysis for defining the boundaries of the putative P-BGC.

An analysis at NCBI BLAST, using the range 3391998 to 3439448 of NZ_OX419519.1 was performed against the “RefSeq Genome Database”, limited to “Actinobacteria (taxid: 201174)”, and using the program “megablast” with default parameters. The graphical summary of the results is shown in panel **A**. The gene cluster, as delimited by synteny analysis with *Kitasatospora cineracea* DSM 44780 is framed in a blue box. All sequences that provided coverage of the BGC (and not just for the homologues of the highly conserved *cysCDN* genes for sulfur metabolism to the right of the 3409997 mark) originated from whole genome sequencing of microorganisms classified as *Streptomyces* or *Kitasatospora* species (table **B**); these alignments were thoroughly analysed, and the segments of the NCBI RefSeq records spanning 50 kb (or to the beginning or end in case the deposited sequence was not long enough) starting from 10 kb downstream of the aldehyde dehydrogenase gene, were downloaded as fully annotated GenBank files. These sequence files, together with the sequence comprising 3391998 to 3439448 of NZ_OX419519, were used for similarity analysis with CLINKER (with the default “Minimum alignment sequence identity” of 0.30). Table **B** shows the strains (with the accession numbers of the sequences used) included in CLINKER analysis, ordered according to CLINKER’s similarity of the PepM encoded by each strain as compared to *K. fiedleri* PepM. **C**. Legend for the colour used in the CLINKER plot to group homologous genes. **D**. CLINKER plot, with BGCs sorted according to, first, phylogeny proximity (*Kitasatospora* strains at the top), and finally the order was adapted to highlight similarity and synteny along the entire BGC and surrounding genes; the plot is aligned at the aldehyde dehydrogenase gene (in red). The BGC from some strains is unfortunately broken in several contigs, what breaks the homology in CLINKER’s plot, but rearranging these contigs would show complete synteny (not shown). It is noticeable that all strains carry the same genes and in the same order, including the gene encoding a LuxR-family transcriptional regulator (in fuchsia pink) and NUDIX-domain encoding gene (in red); curiously the LuxR gene is located to the other extreme of the BGC in the *Streptomyces kanasensis*/*Streptomyces changanensis* group, that seems to also lack the NUDIX-domain encoding gene. Another clear difference is the presence of a gene encoding a putative FAD/NAD(P)-binding protein between the LuxR and NUDIX genes, only in the strains *Streptomyces* sp. NPDC053429, *Streptomyces* sp. NPDC051109 and *Streptomyces* sp. ISL-100. Other small differences might well be due to sequencing errors (that brake the reading frame of a gene and therefore the annotation of encoded protein) or differences in gene annotation (although by using RefSeq database all annotations are expected to be comparable as made with the same or similar pipeline by NCBI).

A phylogenetic analysis was performed with TYGS server to assess phylogenetic relationship among the *Kitasatospora* strains that carry a similar P-BGC to *K. fiedleri*. The full genome assemblies were downloaded from NCBI and submitted as queries to TYGS. Figure in panel **E** shows the strains included in the analysis and the taxonomical judgment provided by TYGS. Panel **F** shows the similarity statistics. Panel **G** shows the full-genome phylogenetic tree. The results indicate that the three strains that show full synteny of the P-BGC and surrounding genes in **D**, *Kitasatospora* sp. NPDC088783, *Kitasatospora* sp. SolWspMP and *Kitasatospora* sp. NPDC090308, actually belong to *K. fiedleri* species. The other two *Kitasatospora* strains that carry an almost identical P-BGC but with different surrounding genes in **D** (*Kitasatospora* sp. Root107 and *Kitasatospora* sp. Root187) fall in a different cluster in the phylogenetic tree, even farer-away than *Kitasatospora cineracea*/*Kitasatospora niigatensis* and *Kitasatospora setae*, strains all that do not carry any P-BGC.

A



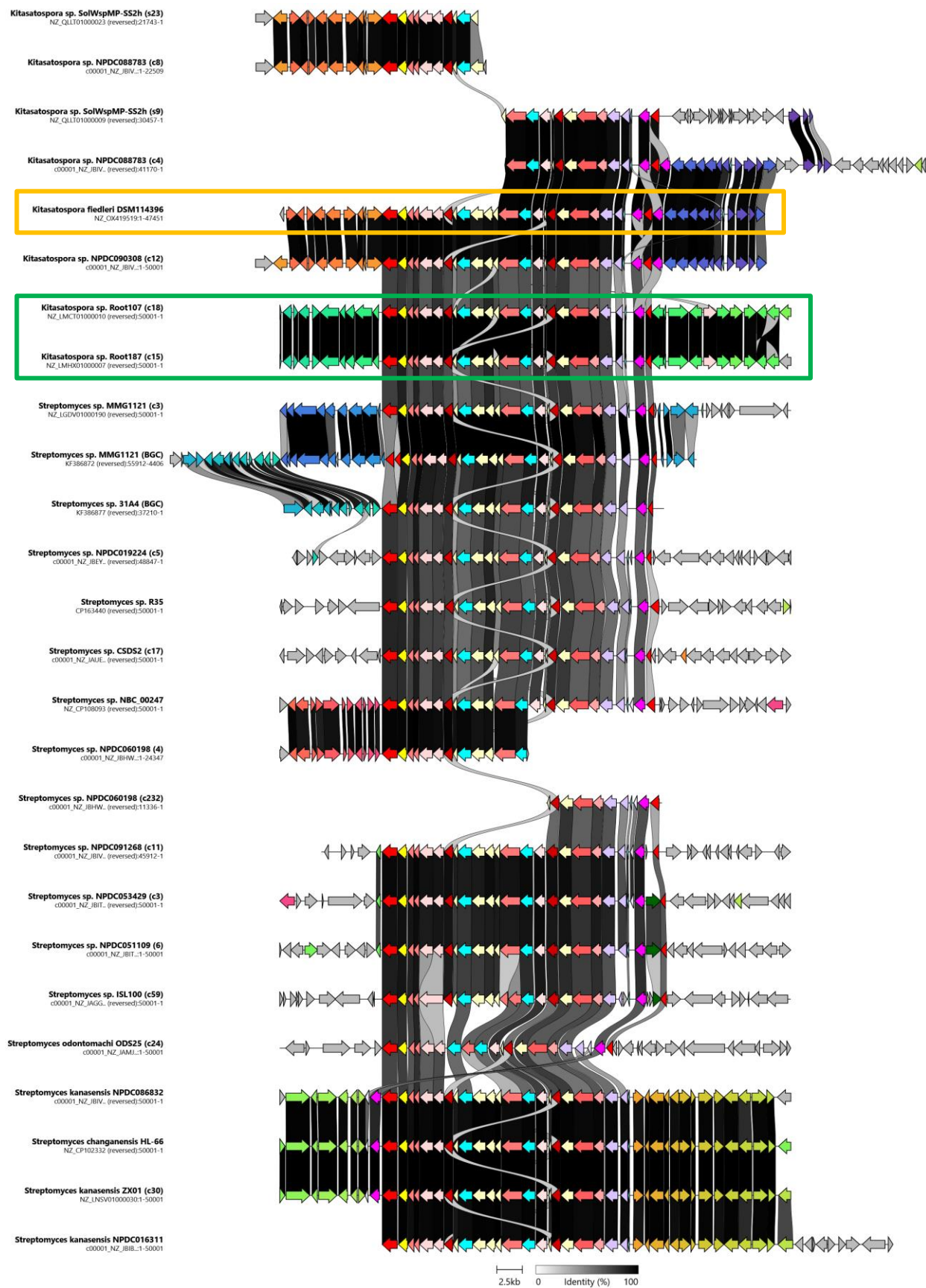
B

Strain source of PepM as compared against <i>K. fiedleri</i> DSM 114396 PepM	Identity	Similarity
<i>Kitasatospora</i> sp. NPDC088783_185_F4.ctg-0008 NZ_JBIVKJ010000008	1	1
<i>Kitasatospora</i> sp. SolWspMP NZ_QLLT01000023	1	1
<i>Kitasatospora</i> sp. NPDC090308_182_E10.ctg-0012 NZ_JBIVXY010000012	1	1
<i>Streptomyces</i> sp. ISL-100 ISL100_59 NZ_JAGGOK010000059	0.87	0.91
<i>Streptomyces</i> sp. NPDC051109_71_F2.ctg-0006 NZ_JBITFF010000006	0.86	0.91
<i>Streptomyces</i> sp. MMG1121 P433contig3.1 NZ_LGDV01000190	0.86	0.91
<i>Streptomyces</i> sp. MMG1121 KF386872	0.86	0.91
<i>Streptomyces</i> sp. NPDC091268_165_H6.ctg-0011 NZ_JBIVVE010000011	0.85	0.91
<i>Streptomyces</i> sp. NPDC053429_373_F9.ctg-0003 NZ_JBITXY010000003	0.85	0.9
<i>Streptomyces</i> sp. R35 CP163440	0.84	0.91
<i>Streptomyces</i> sp. NPDC060198_T5_A10.ctg-0004 NZ_JBHWZJ010000004	0.84	0.91
<i>Streptomyces</i> sp. 31A4 KF386877	0.84	0.9
<i>Streptomyces</i> sp. NBC_00247 NZ_CP108093	0.84	0.9
<i>Kitasatospora</i> sp. Root107 contig_18 NZ_LMCT01000010	0.83	0.9
<i>Kitasatospora</i> sp. Root187 contig_15 NZ_LMHX01000007	0.83	0.9
<i>Streptomyces odontomachi</i> ODS25 24 NZ_JAMJWG010000024	0.81	0.89
<i>Streptomyces</i> sp. NPDC019224_87_D10.ctg-0005 NZ_JBEYCF010000005	0.8	0.89
<i>Streptomyces kanasensis</i> ZX01 contig00030 NZ_LNSV010000030	0.8	0.88
<i>Streptomyces changanensis</i> HL-66 NZ_CP102332	0.8	0.88
<i>Streptomyces</i> sp. CSDS2 NODE_17 NZ_JAUEPB010000017	0.8	0.88
<i>Streptomyces kanasensis</i> NPDC016311 NZ_JBIBSF010000001	0.8	0.87
<i>Streptomyces kanasensis</i> NPDC086832 NZ_JBIVAV010000006	0.8	0.87

C

- QMQ26_RS15720_TauD-TfdA family dioxygenase/QMQ26_RS15770_TauD-TfdA family dioxygenase
- QMQ26_RS15735_sulfate adenylyltransferase subunit 1_cysN
- QMQ26_RS15730_cation-proton antiporter
- QMQ26_RS15725_hypothetical protein/QMQ26_RS15745_adenylyl-sulfate kinase CysC_cysC
- QMQ26_RS15710_acetyl-CoA carboxylase family/QMQ26_RS15715_hypothetical protein
- QMQ26_RS15705_phosphonopyruvate decarboxylase subunit B_ppdB
- QMQ26_RS15700_phosphonopyruvate decarboxylase subunit A_ppdA
- QMQ26_RS15695_phosphoenolpyruvate mutase_pepM
- QMQ26_RS15690_Aldehyde dehydrogenase_adh
- QMQ26_RS15800_hypothetical protein
- QMQ26_RS15795_hypothetical protein
- QMQ26_RS15790_hypothetical protein
- QMQ26_RS15785_histidinol-phosphate transaminase
- QMQ26_RS15780_S9 family peptidase
- QMQ26_RS15775_hypothetical protein
- QMQ26_RS15765_hypothetical protein
- QMQ26_RS15760_aspartate carbamoyltransferase PyrB_pyrB
- QMQ26_RS15755_MFS transporter
- QMQ26_RS15750_inositol monophosphatase family
- QMQ26_RS15740_sulfate adenylyltransferase subunit CysD_cysD
- QMQ26_RS15810_NUDIX domain-containing protein [hydrolase activity]
- QMQ26_RS15805_LuxR C-terminal-related transcriptional regulator_luxR

D



E

Table 2: Identification

- ✔ Your strain 'GCF_003259315.1_ASM325931v1_KitasatosporaSolWspMP-SS2h_genomic' belongs to species *Kitasatospora fiedleri*.
- ✔ Your strain 'GCF_044750705.1_ASM4475070v1_KitasatosporaNPDC088783_genomic' belongs to species *Kitasatospora fiedleri*.
- ✔ Your strain 'GCF_044769485.1_ASM4476948v1_KitasatosporaNPDC090308_genomic' belongs to species *Kitasatospora fiedleri*.
- ✔ Your strain 'GCF_948472415.1_JK4103_genomic' belongs to species *Kitasatospora fiedleri*.
- ✔ Your strain 'GCF_003751605.1_ASM375160v1_Kit.cineracea_DSM44780_genomic' belongs to species *Kitasatospora niigatensis*. Note: ⓘ ⓘ ⚠
- ✔ Your strain 'GCF_003814885.1_ASM381488v1_Kit.niigatensis_DSM 44781_genomic' belongs to species *Kitasatospora niigatensis*. Note: ⓘ ⓘ ⚠
- ✔ Your strain 'GCF_000269985.1_ASM26998v1_Kit.setaeKM-6054_genomic' belongs to species *Kitasatospora setae*.
- ❑ Potential new species detected: your strain 'GCF_001424875.1_KitasatosporaRoot107_genomic' does not belong to any species found in TYGS database. ⓘ
- ❑ Potential new species detected: your strain 'GCF_001429805.1_KitasatosporaRoot187_genomic' does not belong to any species found in TYGS database. ⓘ
- ❑ Potential new species detected: your strain 'Strep_31A4_SRR7783782_contigs' does not belong to any species found in TYGS database. ⓘ

F

Query strain	Subject strain	dDDH (d ₀ , in)	C.I. (d ₀ , in)	dDDH (d ₄ , in) ★	C.I. (d ₄ , in)	dDDH (d ₆ , in)	C.I. (d ₆ , in)	G+C content difference (in %)
'GCF_000269985.1_ASM26998v1_Kit.setaeKM-6054_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	44.5	[41.1 - 47.9]	37.3	[34.8 - 39.8]	42.4	[39.4 - 45.4]	0.15
'GCF_001424875.1_KitasatosporaRoot107_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	23.5	[20.3 - 27.2]	24.1	[21.8 - 26.5]	22.6	[19.7 - 25.6]	2.63
'GCF_001429805.1_KitasatosporaRoot187_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	23.7	[20.4 - 27.3]	24.0	[21.7 - 26.5]	22.6	[19.8 - 25.7]	2.58
'GCF_003259315.1_ASM325931v1_KitasatosporaSolWspMP-SS2h_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	79.6	[75.6 - 83.0]	70.0	[67.0 - 72.8]	80.6	[77.3 - 83.6]	0.41
'GCF_003751605.1_ASM375160v1_Kit.cineracea_DSM44780_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	50.2	[46.8 - 53.7]	42.9	[40.4 - 45.5]	48.7	[45.6 - 51.7]	0.14
'GCF_003814885.1_ASM381488v1_Kit.niigatensis_DSM 44781_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	52.3	[48.8 - 55.7]	42.8	[40.3 - 45.4]	50.4	[47.3 - 53.4]	0.02
'GCF_044750705.1_ASM4475070v1_KitasatosporaNPDC088783_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	75.4	[71.4 - 79.0]	74.3	[71.3 - 77.1]	77.9	[74.4 - 81.0]	0.11
'GCF_044769485.1_ASM4476948v1_KitasatosporaNPDC090308_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	85.3	[81.6 - 88.3]	74.2	[71.2 - 77.0]	86.2	[83.1 - 88.8]	0.25
'GCF_948472415.1_JK4103_genomic'	<i>Kitasatospora fiedleri</i> DSM 114396	100.0	[100.0 - 100.0]	100.0	[100.0 - 100.0]	100.0	[100.0 - 100.0]	0.0
'Strep_31A4_SRR7783782_contigs'	<i>Kitasatospora fiedleri</i> DSM 114396	15.1	[12.3 - 18.6]	21.4	[19.1 - 23.8]	15.3	[12.8 - 18.2]	3.03

G

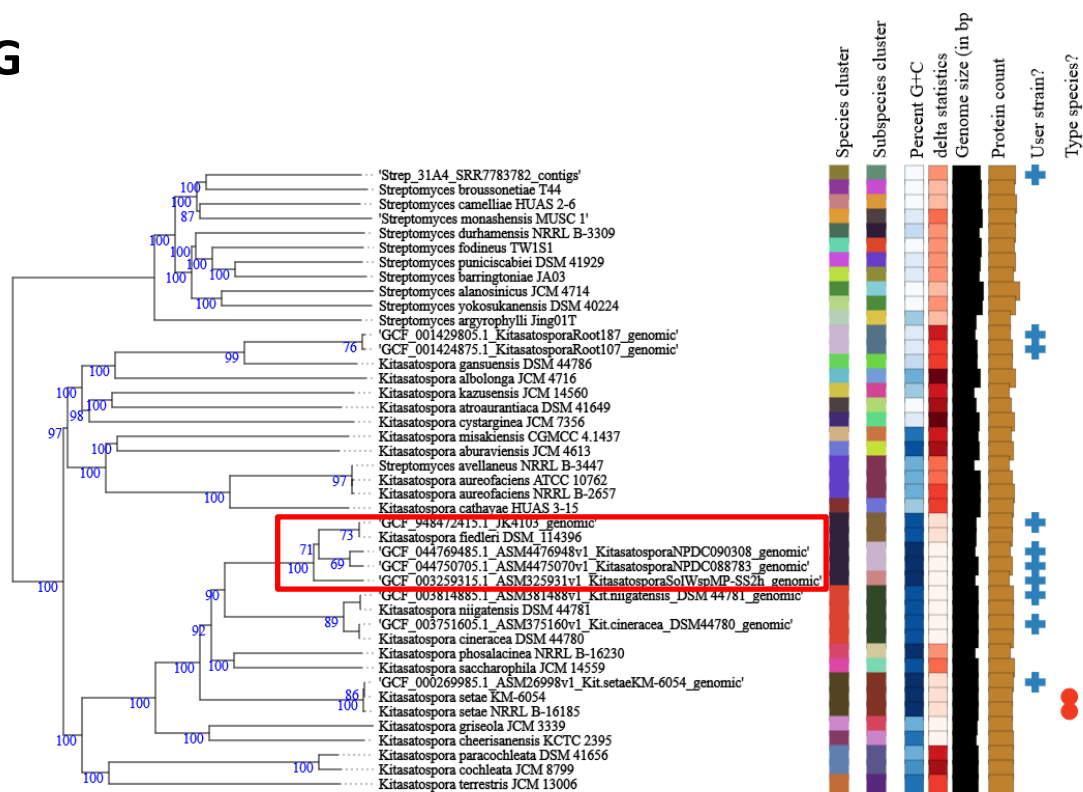


Figure S10. ^{31}P NMR spectra of concentrated supernatants from heterologous expression strains

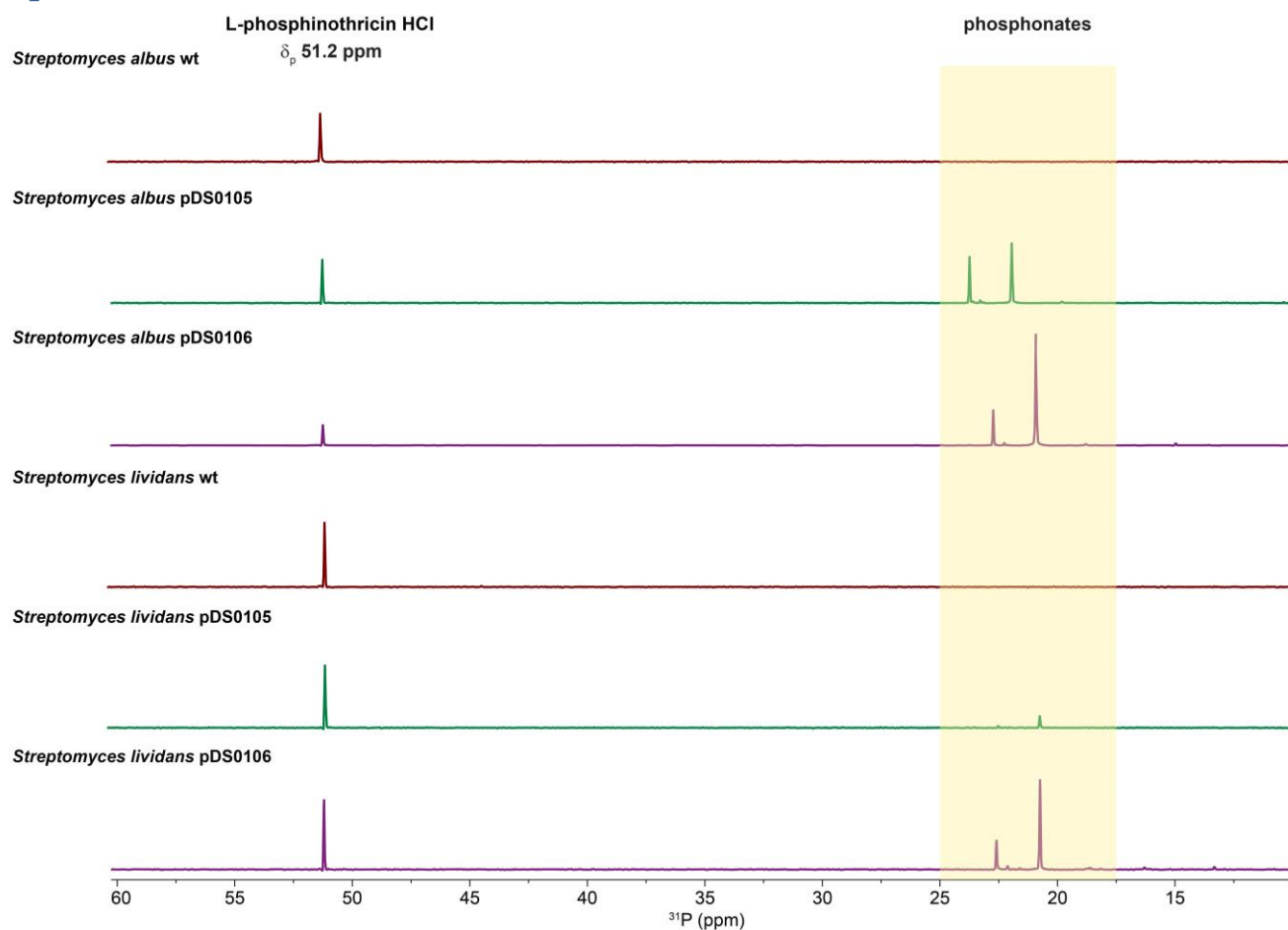


Figure S11. BLAST analysis of amino acid sequences from the *Kitasatospora fiedleri* P-BGC

Each amino acid sequence encoded by a predicted Protein Coding Sequence (CDS) from the antiSMASH predicted phosphonic acid cluster of *Kitasatospora fiedleri* DSM 114396^T was entered in a BLASTp search of the non-redundant protein sequences (limited to taxid2: bacteria) database and the number of proteins in the top 100 hits with sequence identity $\geq 70\%$ (green), $\geq 50\%$ (yellow), and $< 50\%$ (orange) was tabulated revealing regions of high and low homology, respectively.

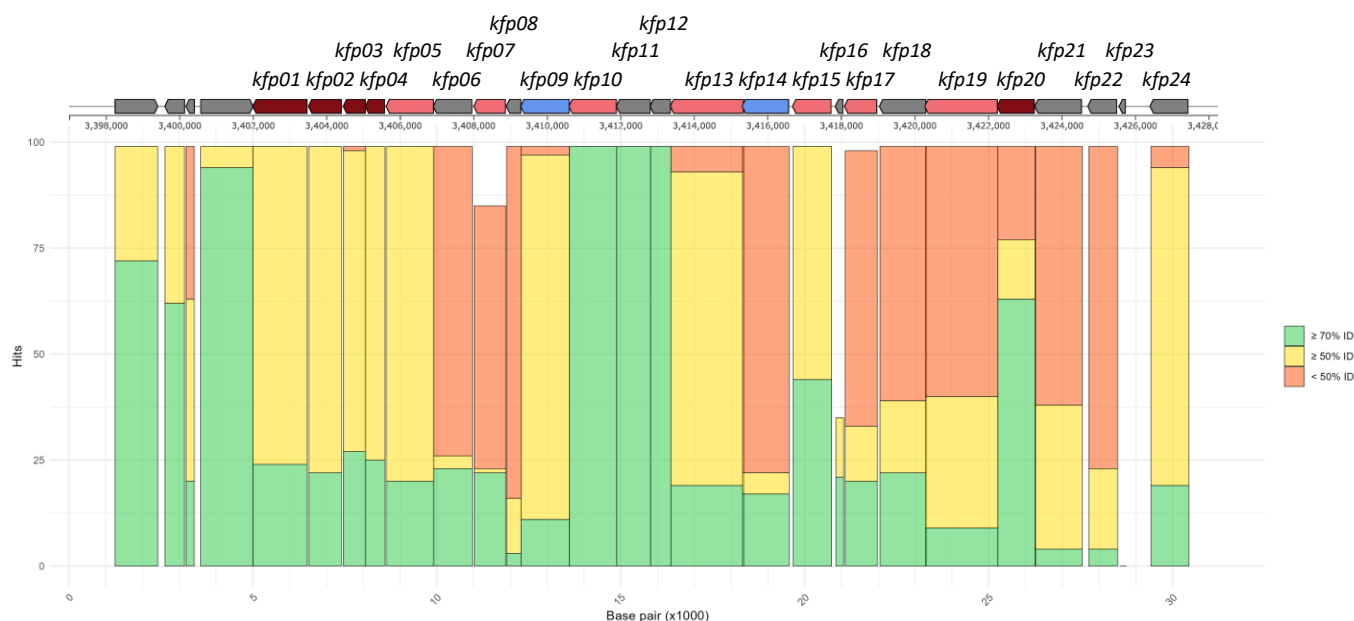


Figure S12. Alignment of aldehyde dehydrogenase enzymes from P-BGCs

(Next page) Alignment of the putative aldehyde dehydrogenase enzymes from the P-BGCs of *Kitasatospora fiedleri* DSM 114396 (Kfiedleri_adh; locus_tag="QMQ26_RS15690" of NZ_OX419519), *Streptomyces* sp. MMG1121 (MMG1121_adh; locus_tag="ADK64_RS20140" of NZ_LGDV01000190), *Streptomyces* sp. 31A4 (31A4_orf24; protein_id="AGZ94408.1" of KF386877), *Streptomyces* sp. NRRL F-525 proven phosphonoacetaldehyde dehydrogenase³ (F-525_fpnG; protein encoded by sequence range complement(102849..104318) of JNXE01000019.1). A second putative acetaldehyde dehydrogenase encoding gene is present in the proximity of PepM in *Streptomyces* sp. NRRL F-525, just upstream of *fpnA* (*orf5* in Supplementary Figure 1 of Freestone et al 2017); we have included this protein in the alignment (F-525_adh2; protein encoded by sequence range 94228..95685 of JNXE01000019.1). On first position, the sequence of the best characterized phosphonoacetaldehyde dehydrogenase enzyme, PhnY from *Sinorhizobium meliloti* 1021⁴. Highlighted in blue or pink background and bold type are the residues important for activity according to the Supplementary Figure 1 of Agarwal et al, 2014. In all cases, all of the catalytic residues (pink) are conserved across all proteins. The residues involved in the phosphate/phosphonate binding pocket (blue) are completely conserved across all proteins proven (FpnG) or proposed to be involved in phosphonate biosynthesis in *Kitasatospora* and *Streptomyces*, and half of the residues also conserved with *S. meliloti*. However, the second putative aldehyde dehydrogenase from *Streptomyces* sp. NRRL F-525, not thought to be involved in phosphonate biosynthesis, is more variable at these important residues.

Smelilo_PhnY --MTNAEVTIAVRHEPMRIAGRLVD--DDRVEVRYPWND-TVVGTVPAGRAEHAREAFA 55
F-525_FpnG MSLSVEIRADRIHRGAHLVGGQHVQG--DALLEKVNPARPGTSLGRIPVADRGVVDSAVR 58
31A4_orf24 MELRRVADRAVADAHNHIAGRWCDARSGRTQENRNPGDLTEVVSRSADSGADDAQAAVA 60
MMG1121_adh -----VADRAVADARNHIAGRWCDARSGRTQEHNRNPGDLTEVVSRSADSATADAQAAVA 54
Kfiedleri_adh MEPR-LTAGPRLAEARNHVAGRWCGAASGRSRDNRNPGDLAETVSRSDSAAADALAAVA 59
F-525_adh2 -----MITYDRLFIAGSWTVPSSPDLLDIRSPHDR-SVIGRAAQALPADVDRSVA 49
: :.* : * :. . . . :.

Smelilo_PhnY IAAAYQP-----KLTRYERQKILLATAEALAAARKEEISDVITLELG---ISKADSLYEVG 107
F-525_FpnG TAHEQLA--AWRKLGSIA RADLVHRLAGLIMERDRGELALLITEEHG---KTRAEDSEVA 113
31A4_orf24 AARRALP--AWQALGSVRRGEIVMRAARLLAERRQFEAETITREQG---KLLSEAFGEVD 115
MMG1121_adh AARAALP--AWQSLGSVRRGEIVMRAARLVAERRQFEAETITREHG---KLLSEAFGEVD 109
Kfiedleri_adh AARAALA--AWRSLGPVRRGEVVLRAARLVAERRAEFAAAITREQG---KLPHEALGEVD 114
F-525_adh2 AARAAFDEGPWPRTAPAERIAVIRRLNALREADAEKIAALISAENGSAWFTLAGQPGLG 109
* * : : : * * . :

Smelilo_PhnY RAFDVFTLAGQMCIRDDGEIFSCDLTPHGKARKIFTMREPLTAISAITPFNHPLNMVAHK 167
F-525_FpnG RSIEIVRFAAGLGRRLLGG---RTLPSDSPDTSSTTRHPIGVVGLITPWNFPLAIPVWK 169
31A4_orf24 RTVDLLEYTAGEGRRLLGG---ATLPADNPRTLALTRREPIGVVGLVTPWNFPLAIPAWK 171
MMG1121_adh RTVDVLEYTAGEGRRLLGG---ATLPADNPRTLALTRREPIGVVGLVTPWNFPLAIPAWK 165
Kfiedleri_adh RTVALLEFTAGEGRRLLGG---ATLPADDPRTLALTRRDPIGVVALITPWNFPLAIPAWK 170
F-525_adh2 RQAGAYLKAAEELGWEET---LAPSDPGSPTRSVLRREPIGVVAVIPWNFSPFSAALAK 165
* :. . . *.* :. : *.* * :

Smelilo_PhnY VAPAIATNNCCVVKPTELTPTMALLLADILYEAGLPPEMLSVVTGWPADIGMEMITNPHV 227
F-525_FpnG LAPALVAGCTVVLKPSPLAPFTAQRLVTLAHEAGVPAGVVNLVHGD-GPTGAALVEHPLV 228
31A4_orf24 VAPALLAGCTSVLKPSPTPLSASLLVDCFVQAGAGGALNLVHGG-REVGEALVADPAV 230
MMG1121_adh VAPALLAGCTSVLKPSPLTPLTASLLVDCFVQAGAGGALNLVHGG-REVGEALVADPVV 224
Kfiedleri_adh VAPALLSGCTAVLKPSPLTPLTATLLVDCFVEAGAGHGVNLVHGG-REVGEALVDHPDV 229
F-525_adh2 VIPALLAGNTVVLKVPSPENSLSMGYLAELLERSNLPEGVISVLPAD-RETSEYLVSHPGV 224
: ** :. *.* : :. : * . :. :. : . :. : * *

Smelilo_PhnY DLVTFGTGSVPVGKLIAN--AHYKRQVLELGGNDPLIILNDLSDDDLARAADLAVAGATK 285
F-525_FpnG KGVSFTGSVDVGRSVQAAAAASLTRTQLEMGGKNAVVL---ADADLEKAADAVAHGAFG 285
31A4_orf24 AGISFTGSVEVGRAIHTAGAPRFLRTQLEMGGKNAALVL---PDADLDEADAITAGAFG 287
MMG1121_adh AGISFTGSVEVGRAIHTAGAPRFLRTQLEMGGKNAALVL---ADADLDEADAITAGAFG 281
Kfiedleri_adh AGVSFTGSVEVGRAIHVAGAPRFLRTQLEMGGKNAALVL---ADADLDRAADAIVAGAFG 286
F-525_adh2 DKIAFTGSTGAGRIASIAGEQLKRVSLLEGGKSAAVIL---DDADVQQAQVGLKFASLM 281
: :****. *.* : * **.* :. : * * :.* : :

Smelilo_PhnY NSGQRCTAVKRILCQESVADRFVPLVLERAKRLRFGDMDRSTD LGTVIHEKAAALFEER 345
F-525_FpnG QAGQRCSATSRAIVQDDVFDEFVARLAARASALRVGRPDDPLTD LGPLINRASLERCLDA 345
31A4_orf24 QAGQRCSATSRRVVDRVHRELVRGLAERATGLRVGTGMDPAARLGPVVSARLEACLNG 347
MMG1121_adh QAGQRCSATSRRVVDRSVHRELVARLAERAAGLRVGTGMDTAAALGPVISAERLEACLNG 341
Kfiedleri_adh QAGQRCSATSRRVVDRVHDLALVRLAARAALRVGPPTDPDARLGPVVSARLEHACLEG 346
F-525_adh2 NNGEACIAQTRVLAPRGYEEVVTALKDLVESLKVGDPNDPDTF IGPMVRPDQQQRVRDY 341
: *.* *.* * : . * : . *.* * : :.* : :

Smelilo_PhnY VMRAAEEGADILYHPPGRS-----GALLPPIVDRVPHQSDLVLEETFGPIIPIVRVPDD 399
F-525_FpnG VSAAVRDGARLVGGTAVVTD--GGYFMAPTVLTGIAADAPLANTEVFGPIILVLRCDRF 404
31A4_orf24 ILLATAEGAVVVTGGHRVTEGVPEGYFMAPTVLDEVRPDSYIAQEEIFGPVLSVIVCDGL 407
MMG1121_adh ILLATAEGAVVVTGGHRVTEGVPEGYFMAPTVLDGVRPDSYIAQEEIFGPVLSVLVCDGL 401
Kfiedleri_adh VRRATADGATVVTGGGRLTEGLPDGYFMAPTVLDLVRPDSHVAQEEIFGPVLSVITCDGP 406
F-525_adh2 IELGIKEGARLVGGPQVPPGLEGGNYVTPTVFADVNSMRIAQEEIFGPVLVVIYPYDDE 401
: . :.* : : * :.* * : . :. * **** : : .

Smelilo_PhnY DDATITLSNSTAFGLSSGVCTNDYRRMQKYIAGLKVGTVNIWEVPGYRIEMSPFGGIKDS 459
F-525_FpnG DEA-VAINNSVRYGMSATLFTASLPLIGRFLTEAEAGMLHVNRPVGGAIPHMPHIGTKES 463
31A4_orf24 DDG-LRIVNSVRYGMAAAVFTRNPSLALDRVEAGMLHVNRPVGGAIPHMPHIGAKDS 466
MMG1121_adh DDG-LRIVNSVRYGMAAAVFTRNPSLALDRVEAGMLHVNRPVGGAIPHMPHIGAKES 460
Kfiedleri_adh EDG-LRIVNGVRYGMAAAVFTRDTSALDALDRIDVGMLHVNRPVGGAIPHMPHIGAKES 465
F-525_adh2 DDA-VRIANDSEYGLSGGVWSADQAHALAVARRLTGTVTVN--GAPFAFDGPFPGFKAS 458
: :. : : * . :.* : : : . * : : * . * * *

Smelilo_PhnY GNGYKEGVIEAMKSFTNVKTFSLPWP-- 485
F-525_FpnG QVGPAECADDAIEFFTELRLTATIGVG-- 489
31A4_orf24 QYGAECSPQVWDFYTQWRTACISY--- 491
MMG1121_adh QYGAECSPQVWDFYTQWRTACISY--- 485
Kfiedleri_adh QYGAECSPQVWDFYTELRSACISY--- 490
F-525_adh2 GIG-REYGAVGLAQYVEYKTTVLPAPVS 485
* * :. : * *

Figure S13. Overview of characterized enzyme functions in phosphonate biosynthetic pathways detected as predicted proteins encoded in *K. fiedleri* pBGC

Some genes of the *K. fiedleri* P-BGC are predicted to be proteins which play known roles in characterized phosphonate biosynthetic pathways. **A** shows the reactions catalyzed by VlpG and VlpF, two ATP-grasp ligases (*kfp05* is predicted to encode an ATP-grasp enzyme) from the valinophos and DHPPA-phosphonate peptide pathway in *S. durhamensis* DSM 40539. VlpG catalyzes the ATP-dependent ligation of L-Val to dihydroxypropylphosphonate (DHPPA) to yield DHPPA-Val ($\Delta vlpG$ strains are deficient in valinophos production). In contrast, VlpF is inactive toward acetyl-Val but instead uses ATP to attach a broad range of L-amino acids onto DHPPA-Val, producing a suite of DHPPA-dipeptides. R = Gly, Ala, Val, Ser, Thr, Leu, Ile, Met, Lys, Arg, Asn, Gln, Trp, Phe, His. Adapted from Zhang et al., 2022 **B** depicts the proposed reaction catalyzed by a TauD dioxygenase (*kfp07* and *kfp17* are predicted to encode TauD/TfdA family dioxygenases) in *Burkholderia pseudomallei* 1710a leading to the synthesis of 2-hydroxy-phosphonoacetate (2-HPnA) from phosphonoacetic acid (PnA). Adapted from Yu et al., 2013. **C** shows the PLP-dependent aminotransferase-catalyzed formation of phosphonoalanine (PnAla). Phosphonopyruvate (PnPy) is transaminated by a PLP-dependent aminotransferase (*kfp20* is predicted to encode the same type of enzyme) from *Streptomyces* sp. NRRL S-515 in the biosynthesis of phosphonoalamides. L-aspartate donates the amino group producing PnAla. Coexpression of PepM and the aminotransferase is sufficient to reconstitute PnAla biosynthesis. Adapted from Kayrouz et al., 2020.

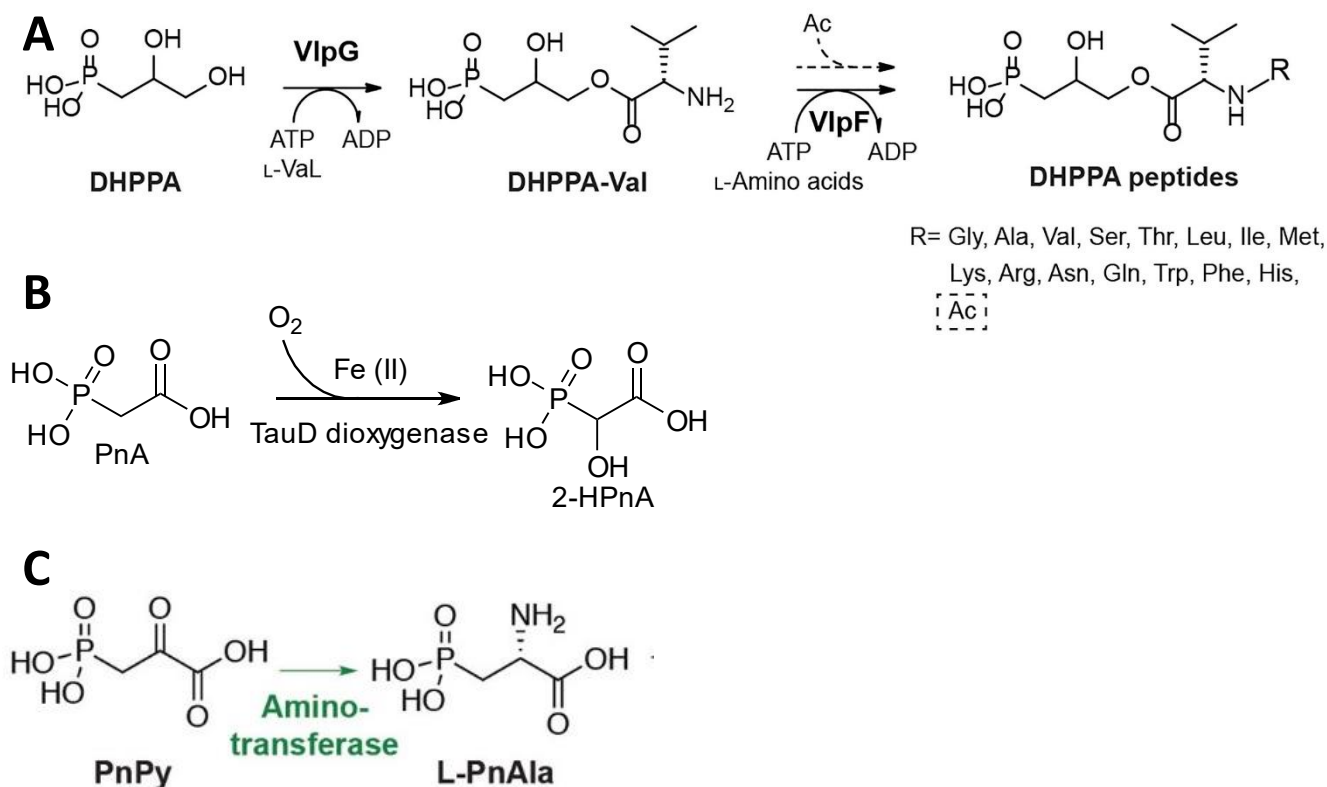


Table S1. Identified potential phosphonate producer strains from the DSMZ and Tübingen collection and known producers of reduced phosphorus compounds included in the analysis

Not all strains have publicly available genome information. (N/A).

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
GCF01								
<i>Kitasatospora atroaurantiaca</i>	DSM 41649	GCF_0078 28955.1	NZ_VIVR01 000001.1	28	MQRVSYPSFRTVLGEHEIVRVAGANHALGGLA EEAGFQAVWSSSLEVSASRGVDPASILSMAEYLE SAANIQKVLRIPIVADCDTGYGGNINVAHMMVH EFEDAGITAVCIEDKLFPMNSFVAGGQTLTDA EFASKIVTAKRAQSTDDMFVIARTEALISGLDVAA ALERCRAVADSGADAVLVHSAKSRDQVLFLE GWDFRCPVIVPTTYPDWHIDEIKAAGSVVVIYA NQGLRATVTALRDTYRAVYRHGDSVVEDSIASV QDIFDLQRLPEWQKLD	286	EDKLFPKMNS	unknown
<i>Streptomyces bikiniensis</i>	DSM 40581, ISP-5580	GCF_0007 16625.1	NZ_JNXJ01 000013.1	30	MLADNEIVRVAGANHALGGLAEEAGFQAVWS SSLEVSASRALPDASILSMTLEYLAAANIQKALRIP VVADCDTGYGGNINVAHMMVHEFAAGITAICIE DKLFPKMNSFVAGGQTLTGTEFADKIRIAKAAQ ATKEMFVIARTEALISGLDVDAALERCRAVADAG ADAVLVHSAKSRDQVVFLEGWDFRCPVIVP TTYPDWHIDEIKAAGSVVVIYANQGLRATVSALR ETYSVYENGDSAVEDSIASVQDVFALQRLA WQKLDV	275	EDKLFPKMNS	unknown
<i>Streptomyces hokutonensis</i>	DSM 102214, R1-NS-10	GCF_0003 76565.1	NZ_BARG0 1000110.1	35	MQRPIYPSFRSVLADNDVVRVAGANHALGGLA EEAGFQAVWSSSLEVSASRGLPDASILSMTLEYL AAANIQKVLRIPIVADCDTGYGGNINVAHMMVH EFEAAGITGLCIEDKLFPMNSFVAGGQTLTGTE EFARKIKIAKEAQASDTEFVIARTEALISGLDVAE LDRCRTYADSGADAVLVHSAKSRDQVVFLE WDFRVPVIVPTTYPDWHIDIRKAGSVVVIYA NHGLRATVSALRDTYRSVYENGDTTALLEGSIASV KDIFALQGLDSWQKLD	286	EDKLFPKMNS	unknown
<i>Streptomyces regensis</i>	DSM 40551, NRRL B-11479	GCA_001 047335.1	LFVR01000 169.1	49	MQRPIYPSFRSVLADNDVVRVAGANHALGGLA EEAGFQAVWSSSLEVSASRGLPDASILSMTLEYL AAANIQKVLRIPIVADCDTGYGGNINVAHMMVH EFEAAGITGLCIEDKLFPMNSFVAGGQTLTGTE EFARKIKIAKEAQASDTEFVIARTEALISGLDVAE LDRCRTYADSGADAVLVHSAKSRDQVVFLE WDFRVPVIVPTTYPDWHIDIRKAGSVVVIYA NHGLRATVSALRDTYRSVYENGDTTALLEGSIASV KDIFALQGLDSWQKLD	286	EDKLFPKMNS	unknown
<i>Streptomyces regensis</i>	NRRL WC-3744	(N/A)	KF594335 Region on contig edge.	(N/A)	MQRPIYPSFRSVLADNDVVRVAGANHALGGLA EEAGFQAVWSSSLEVSASRGLPDASILSMTLEYL AAANIQKVLRIPIVADCDTGYGGNINVAHMMVH EFEAAGITGLCIEDKLFPMNSFVAGGQTLTGTE EFARKIKIAKEAQASDTEFVIARTEALISGLDVAE LDRCRTYADSGADAVLVHSAKSRDQVVFLE WDFRVPVIVPTTYPDWHIDIRKAGSVVVIYA NHGLRATVSALRDTYRSVYENGDTTALLEGSIASV KDIFALQGLDSWQKLD	286	EDKLFPKMNS	hydroxynitrilaphos ⁵
<i>Streptomyces</i> sp.	TUE 18	(N/A)	(N/A)	34	MQRSHPSFRSVLADNEIVRVAGANHALGGLA EEAGFQAVWSSSLEVSASRALPDASILSMTLEYL AAANIQKALRIPIVADCDTGYGGNINVAHMMVH EFEAAGITAICIEDKLFPMNSFAAGGQTLTGTE	286	EDKLFPKMNS	unknown

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ³ NS)	phosphonate product
					FADKIRTAKAAQATQDMFVIARTEALISGLDVDA ALERARAYADAGADAVLVHSHKVKTRDQVVGFL GWDNRVPVIVPTTYPDWIDEIKQAGVSVVIY ANQGLRATVSALRETYRSVYETGDSTAVENSIA VQDIFALQGLEAWQKLDV			
<i>Streptomyces xanthophaeus</i>	DSM 116597, 11.9	(N/A)	(N/A)	33	MQRSYPSFRSVLAENEIVRVAGANHALGGLAE EAGFQAVWSSSLEVSASRALPDASILSMTEYLE AANIQKALRIPVADCDTGYGGNINVAHVMHE FEAAGITAICIEDKLFPMNSFVAGGQTLTGKEF ADKIRIAKAAQATQEMFVIARTEALISGLDVDA LERCRAYADAGADAVLVHSHKVKTRDQVVGFL WDFRVPVIVPTTYPDWVDEIKQAGVSVVIYA NQGLRATVSALRDTYRSVYETGDSTAVEGSIASV QDVFALQRLAEAWQKLDV	286	EDKLFPKMNS	unknown
GCF02								
<i>Streptomyces</i> sp.	NRRL S-481	GCF_0007 21955.1	NZ_KL5851 64.1	29	MLKRSYPSFRSVLAHDVVRVLAGAHHALSGVLA QAGFQAVWSSSLEVSASRCLPDASILSMTEYLE AAANIQKALDIPVADVDVTGYGGNINVAHVMH EFEGAGITAVCMEDKLFPMNSFANGDQTLTLD AAFTGKIQTAKAVQSTEDFFLIARTEALISGAGVD EAVSRCNAYADAGADAVLIHSHKARTNEEVLAFLD RWDNRVPVIVPTTYPDWIDEVRAAGVSVVIY ANQGMRAALSAMRNTYRAIYEAGHTLLESEIAS VRDIFDLQRMADWQKLD	286	EDKLFPKMNS	phosphono-cystoximate ⁶
<i>Streptomyces</i> sp.	I6	GCF_0037 25745.1	NZ_RHDP0 1000001.1	29	MLKRSYPSFRSVLAHDVVRVLAGAHHALSGVLA QAGFQAVWSSSLEVSASRCLPDASILSMTEYLE AAANIQKALDIPVADVDVTGYGGNINVAHVMH EFEGAGITAVCMEDKLFPMNSFANGDQTLTLD AAFTGKIQTAKAVQSTEDFFLIARTEALISGAGVD EAVSRCNAYADAGADAVLIHSHKARTNEEVLAFLD RWDNRVPVIVPTTYPDWIDEVRAAGVSVVIY ANQGMRAALSAMRNTYRAIYEAGHTLLESEIAS VRDIFDLQRMADWQKLD	286	EDKLFPKMNS	unknown
GCF03								
<i>Kitasatospora setae</i>	DSM 43861, KM-6054	GCF_0002 69985.1	NC_016109 .1	41	MLRVNHQSFRVLAESDVVRVAGAHHSALGYLA QAGFQAVWSSSFEVSASRCLPDASILSMTEYLE AAANIQKALSIPVADCDTGYGDNINVAHVMH EFEGAGITAVCIEDKLFPMNSFVSGQALLDTA GFAAKIRTAKNAQHSSDFFVIARTEALISGLDVTQ ALERCRAYADGADAVLVHSHKQRTKDQVMAFL DGWDGRVPVIVPTTYPDWIDEVRAAGVSVVI YANQGLRASVSLRDYGAIVRDGDTTGIEESIAS VGDLFELQRLAEWQKLG	286	EDKLFPKMNS	unknown
GCF04								
<i>Kitasatospora purpeofusca</i>	DSM 40283, NRRL B-1817	GCF_0007 18025.1	NZ_JODS01 000049.1 Region on contig edge. supercluster	45	MSPTPHSYPSFRSVLADHPLVRIAGAHSAALGGVI AEQVGFQAVWASSFEISAARCLPDASILSMSEYL DAAHIQKALSIPVADCDTGFNGSMNVAYM VAEYEQAGITAVCMEDKIFPMNSFAADHSL DVNAFAHKIETAKKSQRSEDFVIARTEAMISGL GVDEALKRCVADAGADAVLVHSHKQATKDEVL SFLDQWDDRLPVVPTTYPDWHADEARAAGV SAVIYANQGLRATITSLRSALGSIYETGHSALHEDS IAGVKDFALQRLDQWQETA	288	EDKIFPMNS	unknown
<i>Saccharothrix</i> sp.	ST-888	GCF_0009 55975.1	NZ_JYJF010 00012.1	50	MSLNIHPSHPSFRSVMAEHLPTRIAGAHSAALGG VIAEQVGFQAVWASSFEISAARCLPDASILSMTD YLEAAANIQKALTVPVADCDTGFNGSMNVAY MVAEYERAGITAVCMEDKIFPMNSFAAAHTL LDAGDFAHKIETAKKQRSEDFVIARTEAMISGL	289	EDKIFPMNS	phosphonothrixin ⁷

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
			Region on contig edge.		GVDEALKRCTTYADAGADAVLVHSHKATKDEVLEFLEQWDRNRPVVVPTTYPDWHADAEHAAGVSAVIYANQGLRATITSLRTALGSIYQTGHSALHLEDSIAEVKDVFAQLRQLGQWQEF			
<i>Streptomyces</i> sp.	WM4235	GCF_001279725.1	NZ_LGDE01000429.1 Region on contig edge.	40	MIRNSYPSFRQVLADNPLTKIAGHSLGGRIAEQSGFQAVWASSFEISAARCLPDASLLSMTDYLEAANIQKALSVPVVADCDTGFGNSMNVAYMVAEYESAGITAVSIEDKIFPKMNSFAAADHTLLDVSAFAHKIETAKKAQRTDDFFVIARTEAMISGLGVDEALKRCNAYADAGADAVLVHSHKQTKDQVLEFLAHWDDRLPVVPTTYPDWHADARAAGVSAVIYANQGLRATITSLRSTYSIYETGHTSHLEDSISGVKDVFDLQQLDEWQQLGA	286	EDKIFPKMNS	phosphonothrixin ⁸
<i>Streptomyces</i> sp.	TUE 21470	(N/A)	(N/A) supercluster	42	MSPTPHSYPSFRSLADNPLVRIAGAHSLGGVIAEQVGFQAVWASSFEISAARCLPDASLLSMSEYLDAAAHIQKALSVPVVADCDTGFGNSMNVAYMVAEYEQAGITAVCMEDKIFPKMNSFAAADHSLLDVNAFAHKIETAKKSQRSEDDFFVIARTEAMISGLGVDEALKRCAVYADAGADAVLVHSHKQTKDEVLSFLDQWDDRLPVVPTTYPDWHADARAAGVSAVIYANQGLRATITSLRSLGSIYETGHSALHLEDSIAGVKDVFAQLRQLDQWQEF	288	EDKIFPKMNS	unknown
GCF05								
<i>Streptomyces aureus</i>	NRRL B-2808	GCF_001418135.1	KC733813.1 Region on contig edge.	(N/A)	MTASRPPQQRNDPPDGAARRLAALAPRLTRAMGAHSPLSARLAEAGFDVWSSGLEISAAA GVPDANILAMGECLDAAAGLASSVDLPVLADCD SGFGNNVNIHVMVRSYARGVAGVICEDKQFPK LNSFVEGNQDLAPLDDFAAKIRGASETRTDMVV VARLEALISGQGMAEALRRADVYERAGADALLIHSKRKDPPEEVFAFREAYRGDLPVIVPTTYNQVTV EEEQRFAMAIYANQALRSSIRAMRETLTRIMR DGTTLNVEPELAPLKEIFDLQRMPLQEQERY ESLGRLAEAAQ	311	EDKQFPKLNS	phosacetamycin ⁹
<i>Streptomyces</i> sp.	A41	(N/A)	(N/A)	37	MTESRSPQQRNERTPSAAQRLRAALATPRLTRAM GSHSPLSARLAEAGFDVWSSGLEISATAGVPD ANILAMGECLDAAAGLASAVDIPVLADCDSDGFG NVNNVIHVMVRYESRGMAGVICEDKQFPKLS FIEGNQDLAPLDDFAGKIRAAATETRTDMVVVARL EALISGQGMAEALRRADVYERAGADALLIHSKRK DPPEEVFAFREAYRGHLPVIVPTTYNQVTV EEE QRGFAMAIYANQALRSSIRAMRETLTRIMRDGT TRNVEPDIAPLKEIFDLQRMPLQEQERYESV GRELAEAAK	309	EDKQFPKLNS	unknown
<i>Streptomyces</i> sp.	A4-2	(N/A)	(N/A)	39	MTESRSPQQRNERTPSAAQRLRAALATPRLTRAM GSHSPLSARLAEAGFDVWSSGLEISATAGVPD ANILAMGECLDAAAGLASAVDIPVLADCDSDGFG NVNNVIHVMVRYESRGMAGVICEDKQFPKLS FIEGNQDLAPLDDFAGKIRAAATETRTDMVVVARL EALISGQGMAEALRRADVYERAGADALLIHSKRK DPPEEVFAFREAYRGHLPVIVPTTYNQVTV EEE QRGFAMAIYANQALRSSIRAMRETLTRIMRDGT TRNVEPDIAPLKEIFDLQRMPLQEQERYESV GRELAEAAK	309	EDKQFPKLNS	unknown
<i>Streptomyces</i> sp.	TUE 3678	(N/A)	(N/A)	36	MNKPNSQPSAEALRAALHAERPARAMGAHSP LSARLAEAGFEVWSSGLEISAAAGVPDANILA MPQCLEAAASLAESVSPVLADCDSDGFGNVNN VIHVMVRYEARGVAGVICEDKQFPKLSFIEGN QDLAPVDDFAAKIRAAETVRRDLVVVARLEALIS GQGMAEALRRADVYERAGADALLIHSKLADPGE VFAFREAYTGDLPIAIPITYHQVTIEDLRERGF MAIYANQALRSSIRAMRATLTKIQDGSHTGVEE	303	EDKQFPKLNS	unknown

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
					DIAPLKEIFDLQRMPQMLEQQARYEALGRDLAE AGR			
GCF06								
<i>Streptomyces kutzneri</i>	DSM 40907	GCF_0322 48635.1	NZ_JASTTIO 1000003.1 Region on contig edge.	34	MFKPVSYSPFRSLVAQRRLSQAVRVAGAH DAL GGR LAQAAGFD AVWASSLEVSAARCLPDASVLT MTEYLEAAAHTQKALGIPVVADVDVTGYGNNLN VAH MVHEYEAAGITAVCMEDKLPK MNSFVAT SHTLLDTEYCSKVRVAKNAQRGEDFFFIARTEAL IDGLGVEVALERCHAYVDAGADAVLIHKSRTKD QVLEFLQGWDRAPVVVVPTTYPDWHADDAV KDG VSTIIYANQGLRATV SALRD TYASILSHGETT RLEDKIASVKDVFALQRLSDWQELESA	292	EDKLFPKMNS	phosphonoalamide A-D ¹⁰
<i>Streptomyces resistomycificus</i>	DSM 40133	GCF_0015 14265.1	NZ_KQ949 005.1	30	MLKSALHPSFRSLVAARRAAGSAVRVAGAH DAL GGR LAEAAGFD AVWASSLEVSAARCLPDASVLT MTEYLEAAAHTQKALGIPVVADVDVTGYGNNLN VAH MVREYEAAGITAVAIEDKQFPK MNSFVATS HTLLD TDTFASKLRVAKQAQRTEDFFVIARTEALI DGLGVEVALERCHAYVDAGADGVLVHSKKPTNE QVLAFLAEWQDRAPVVVVPTTYPDWHIDDAVK AGVSTVIYANQGLRATIRALRETSTIVDQGATTE LEDRIASVKDIFALQSLAEWQELESA	292	EDKQFPKMNS	unknown
<i>Streptomyces</i> sp.	B-2790	GCF_0416 95405.1	NZ_JBAFS W0100000 02.1 Region on contig edge.	47	MLKSTPHSPFRTVLTAARRAAGSAVRVAGAH DAL GGR LAEAAGFD AVWASSLEVSAARCLPDASVLT MTEYLEAAAHTQKALGIPVVADVDVTGYGNNLN VAH MVREYEAAGITAVAIEDKQFPK MNSFVATS HTLLD TETFSKLRVAKQAQRTEDFFVIARTEALI DGLGVEVALERCHAYVDAGADGVLVHSKKPTNE QVLAFLAEWQDRAPVVVVPTTYPDWHIDDAVK AGVSTVIYANQGLRATIRALRETYGTIVERGDTTE LEDRIASVKDIFALQSLTDWQELESA	292	EDKQFPKMNS	phosphonoalamide A-D ¹¹
<i>Streptomyces badungensis</i>	DSM 114471, 195335CR	(N/A)	(N/A)	30	MFKPVSYSPFRTVLGERAAAKAVRVAGAH DAL GGR LAQSAAGFD AVWASSLEVSAARCLPDASVLT MTEYLEAAAHTQKALGIPVVADVDVTGYGNNLN VAH MVHEYEAAGITAVCMEDKLPK MNSFVAT AHTLLD TDAFCSKIRVAKNAQRGEDFFFIARTEAL IDGLGVEVALERCHAYVDAGADAVLIHKSRTKD QVLEFLREWKDRAPVVVVPTTYPDWHADDAFK DGVSTIIYANQGLRATV SALRD TYASILSHGDTTH LEDKIASVKDVFALQRLSDWQELETA	292	EDKLFPKMNS	unknown
GCF07								
<i>Salinispora pacifica</i>	DSM 45543, CNS-863	GCF_0003 83995.1	NZ_KB9130 22.1	24	MSDSNVLVRTGQSKAAALRALLSADHPVRAVG AHDALTAKLVEQAGFD AVWSSSFEISASHGLPD ASIVTMSQYLASSEAMD LGVTIPVIADCDSGYGG PLHTAYAVQRFERAGIAAVCIEDKLPK MNSFAD VTQELVTAEEFAMKIKAAEVQNDPDMVIART EALIAGLSITEALDRGHAYVEAGADAVLVHKS KR PDEVLEFASRWSDSPLVAVPTTYSISEAALGA AGFRIVYANQGLRAAVRGVQETLQELSRAGCA QAVSERIAPMHEVFALQGMRAGRFRDKP	296	EDKLFPKMNS	unknown
<i>Streptomyces chrestomyceticus</i>	DSM 40545, NBRC 13444	GCF_0038 65135.1	NZ_BHZC01 000001.1	45	MSENATTTNKSARLRELLAGPRPVAAGAH DGL SAKLVEQAGFD AVWCSSFEVSASYGLPDASLVT MTQFLAAAEAMDALIDIPVIADCDTGFGGPLNV AFAVERYERAGIAAMCIEDKLPKINSFADAGQD LLPTKEFALKIEAGKQTQKDERFLLIARTEALISGQ GVPEALERAHAYADAGADAVLVHKSRRPDDIL ELGDWDRDPLVAVPTTYSVEEKALFDAGYR LVIYANQGMRAAVKNMREVLGKLRVEGRAESV DADIATMPEIFALQGMATAAFRTAP	291	EDKLFPKINS	unknown

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
<i>Streptomyces monomycini</i>	DSM 41801, NRRL B-24309	GCF_000715845.1	NZ_KL571064.1 Region on contig edge.	45	MSENTTTNKSALLRELLAGPRPVAAGAHGDLGSLAKLVEQAGFDVWCCSFEVSASYGLPDLASLVTMTQFLAAAEAMDADIDPIVADCDTGFGGPLNVAF AVERERAGIAAMCIEDKLFKINSFADAGQDLLPTKEFALKIEAGKQTKDERFLIARTEALISGGGVPEALERAHAYADAGADAVLVHKSRRPDDILELGDADWRDPLVAVPTTYASVEEKALFDAGYRLV IYANQGMRAAVKSVREVLGKLRAEGRAEAVDA DIATMPEIFALQGMATAFRTAP	290	EDKLFPKINS	argolaphos ⁶
GCF08								
<i>Streptomyces alboniger</i>	DSM 40043, ATCC 12461	GCF_008704395.1	NZ_CP023695.1 supercluster	30	MTVVSNSYVETSSRALVLRDAFESGRVLKVGAGHDGLSARLAMEAGFDVWASGLEISAAQGLPDVSLGMAEYLAGATAMQQAVSIPVADCDTGF GGSLNAAAYTMRRYEGAGVAGICIEDKIFPKRNSF LDAGQKLLTDEFSGKLEAAKKAQARPETLLIART EAFICGMGVDEALRRCHQYVDAGADAVLVHKS AEAGEVVSFMRQWQHRAPVIVPTTYADFSVQ EAQEAGISMVIYANQGMRAVSKAVRDTWAVVL AEGSTASVEPHIATVKDIFSLSGIDHWLGDDK	295	EDKIFPKRNS	unknown
<i>Streptomyces luridus</i>	NRRL 15101	(N/A)	GU199252 Region on contig edge.	(N/A)	MTIASYGPSTLPGSRAATLRDAFANGRLVRVAG AHDGLSARLAEAGFDSVWASGLEISAAHGLPDVSLGMAEYLSAAVAMQRSVSVPVADCDTGF GGVLNAAAYTMRRYEEAGVAGVICIEDKIFPKRNS FVNSGQKLLDVDEFGRKLEAAKRAQVSSDTVLIA RTEAFICGLGLEALTRCHHYVDAGADAVLVHSAVDSGEVVSFMRQWQHRAPVIVPTTYADFSAEAEQEAGISMVIYANQGMRAAIAIRSTWATVLAEGSTAPVEPRIASVKDIFALSGMDQWLDLDR	296	EDKIFPKRNS	dehydrophos ¹²
<i>Streptomyces</i> sp.	TUE 3997	(N/A)	(N/A) supercluster	37	MTITSSSESTTRTKSTVSSPRAAALREAFADARLV RTAGAHDLGSLARLAEAGFDVWASGLEISAAANGLPDVSLGMAEYLAVAGAIQRSVSLPIADCDT GFGGVLNAAAYTMRRYEEAGIAGICIEDKIFPKRN SFVNAGQKLLAADEFGRKLLQAAKRAQLSADTVLI ARTEAFICGLGLEEALSRLCHLYVDAGADAVLVHSAVDSGEVVSFMRQWQHRAPVIVPTTYADFSAT QAREAGIAMVIYANHGMRAAIAIRSTWADVL AKGSTADLEPRIATVKDIFALSGMDQWLETDR	302	EDKIFPKRNS	unknown
GCF09								
<i>Kitasatospora fiedleri</i>	DSM 114396 ^T , TUE 4103	GCF_948472415.1	NZ_OX419519.1	25	MSKSRTLRLDGLNGSEVIRLVGAHSALSAGLQGD AGFEAIWASGLEISASRALPDANVLSMSECLEAA AQIADAVDPVPLADCDSGFGGVGNVAHVMVRSY QARGLAGVICIEDKQFPKLNSFVEGHQDLAPIGDF AAKITAAKEARTGEDFVVVARIEAFIAGAGLDEAL RRATVYEAAGADALLHSLKSTPEEVFAFRAAYTG ALPVIVVPTTYPQVTAELTERRFGGVIANQGL RAAISAMRDVLGQIGAAGSTHAVEGSIATLKDVF ALQNVLDALLARQERHDLTAQYAALG	297	EDKQFPLNS	unknown
<i>Streptomyces</i> sp.	31A4	(N/A)	KF386877.1 Region on contig edge.	(N/A)	MSNTRTLRLDGLNGSAITRLMGHLSAKLGEAGFEAIWASGLEISAAALPDANILSMAECLQAA TEIAGAVDIPVPLADCDSGFGGVGNVHVMVRSY ASGVAGVICIEDKQFPKLNSFVEGNQDLAPVGDF AAKITAAKEARREDFVVVARIEALIAGAGMEEA LRRARVYEAAGADALLHSLKSTPEEIAFRAAYD GALPLIVVPTTYPQVTAELAERFGGAVIANQGL RASISAMRDVLARIDAAGSTYGVESIAGLKDVF ALQKQVDELLARQERHDLTAAYASLG	297	EDKQFPLNS	unknown

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
GCF10								
<i>Streptomyces viridochromogenes</i>	DSM 40736, TUE 494	GCF_000158955.1	NZ_GG657757.1 supercluster	33	MNATERPGSDGTGSPESVGSRLNLLHGPCTCQ LMGVHDGLSARIAVAEGFEALWASGLCMSTAR GVRDSDEASWTELLTVGMTTEAPGAPVLVD GDTGYGNFNTARRFAARAERVGAAGVCFEDKV FPKMNSFFGDGHQLAPIGEFSKGKACKDTQRD PGFVVVARTEALISNLPMEELTRAHAYVEAGA DGLFIHSMSTPQQIAEFMRQWDGSAPILAPTT YHRPSLDDFAALGIAGCIWANHSMRAAFSAMR DVCQQRADRGIFGVEERVAPLKEIFGLLDYESLE QDENRYTQAPDLAPVQG	313	EDKVFPMKNS	phosphinothricin tripeptide ^{13,14}
<i>Streptomyces mooreae</i>	DSM 41527, ATCC 21705	GCF_031845725.1	NZ_JAVRFE010000017.1 supercluster	39	MNATEQAASGDRGTTTSAGGRLRYLLHAPGAC QLMGVHDGLSARIAVAEGFEALWASGLCMSTA RGVRDSDEASWTELLTVGMTDAVPGVPVLV DGDGTGYGNFNTARRFAGRAERVGAAGVCFEDK VFPKMNSFFGDGHQLAPVAEFCGKIRACKDAQR DPDFVVVARTEALISKLPMEELDRAAAYAEAGA DALFIHSMSTPQQIATFMERWEGSTPVLAPTT YHTPSVDDFAALGIAGCIWANHSMRAAFAMR DVCQQRIRDRGIYGEDQVAPLKEIFGLFDYEGLE KDENCYTQAPDLAAVQG	313	EDKVFPMKNS	phosphinothricin tripeptide ¹⁵
GCF11								
<i>Goodfellowiella coeruleoviolacea</i>	DSM 43935	GCF_024171785.1	NZ_JAMTC K010000002.1	51	MDSTRPAQPPSRSLRAMLAPEISFLMEAHN GLSARIAQAHGFPGIWASGFAISTALGVDRSNE STKELLDTVAFMVDATTVPVVDGDTGYGNFNN ARRLRQLGRLGVSACLEDKLFPKTNSFLGTGQ PLADEREFCGKIRACKDSQLDAEFCVVARVEALV SGRGLTEALHRAESYRRAGADAVFIHKSQQDGR EVLDFAREWAGRCPLVITPTTYHVGVDFAFERA GVAMLIWANQNMRAAVLAMRQVCQSIAANR GLTAVEPALVSVQEVDFLDYDELDAATLRYSSW HGDETTAGAGP	309	EDKLFPKTNS	unknown
<i>Streptomyces rimosus</i>	DSM 40260, ATCC 10970	GCF_000331185.2	NZ_CP048261.1 supercluster	46	MGDTGSGIQREDLMHNNHHTPTAKTTMMRE LLRSRLGFLMEAHNGLSARIVEDVGFPGIWASG FSISTALGVDRSNEVSAGEVLDTVAYMADATRIPI MVDGDTGYGNFNNARRFVRQLCRLGVAGVCIE DKLFPKTNSFIGDRQPLADTDEFRGKIAACKDSQ TDDDFCVIARVEALVSGFSLAEALGRAEAYRQAG ADAVFIHSKRKDGKEVLAFAREWGRRSPLVITPT TYHRSVGVDTFERAGISTLIWANQNMRAASMRA MREVSEEVFKRRVSDVEQDLVPVQEVDFLDY GELDDASRRYLPADPAPASRRDAS	323	EDKLFPKTNS	unknown
GCF12								
<i>Pantoea ananatis</i> (1)	LMG 5342(-1)	GCF_000283875.1	NC_016816.1	9	MIKKLIAEKTGLFIEAHNPLSALIAKAEQTNSEG RIVKFDGIWSSSLTDSASRGIPDNETLALSSRLNI ADIRNVTDMPIMDADTGGKPEHFSYVYKRMIN NGVNGVIEDKTGLKKNLSFGTEVEQTLADINDFS EKIKRGKSAYVIDDFMIARLESLIAGFDVEHALER ADAYVEAGADGIMIHSCKKTPDEVFLSTKFRKK YPSVPLICVPTTYSATSNRELSEAGFNVIYANHM LRAAYKAMENVSKILRYGRTAEIEKSCMSVKEIIS LIP	284	EDKTGLKKNS	pantaphos ¹⁶
<i>Streptomyces thermoviolaceus</i>	DSM 116655, SFG18	(N/A)	(N/A)	23	MSISTIGNETRLTALRESLRGEPVRIEVHSPALAA VIAEHAQKGPDREFHGFWSSSLTDSALRGLP DIELDMEVRLSWIDQIFSVSTLPLVMDGDTGG QTLHFEYLVKAMERRGVSAVVEDKCGEKNRSL	306	EDKCGEKNRNS	unknown

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
					PGEGLHTMAPVDEFCDKISRGAQSSDAFMII ARLEGLITGLSRQEILDRAGAYVAAGDGLVLHS RSSDEAPLLDLARELRRRHPDTPLVAITPAYPSVT EEELHAAGFNMIYANQMLRAATRAMEEVSHRI LDHGRALEAGEVCIETSKLLTMPDDKRPIVRAPR N			
GCF13								
<i>Streptomyces doudnae</i>	DSM 41981	GCA_031 845505.1	NZ_JAVRES 010000001.1	38	MTATGRTVGRTAALRHLLERAEITFLMEAHNGL SAKLVEQAGFEGIWASGLSIAAALGVRDSNEAS WTQVLEVAEFMSDATRIPLLDDGTGYGNFNSV RRLVRKLEQRGVAGVCVEDKLPKTNFSFVRGGA QPLADAEFAGKIGAAKASQDQDFVVARTEA LIAGLGAEALRRAELYRRAGADAILVHSARADA GEVRAFKAEWGDRLPVVIVPTAYRTPTVEFAED GISTVVWANHLMRSLRAMQITASTIFAEQHLR NVEGTVAPLAEVFALQGEDELAEEARWLPRRN TPSR	303	EDKLFPKTNS	unknown
<i>Streptomyces hintoniae</i>	DSM 41014	GCA_031 845745.1	NZ_JAVRFF 010000014.1	41	MTAPGRTTSRAAALRHLLDRPELSFLMEAHNGL SAKLVERAGFEGIWASGLSIAAALGVRDSNEAS WTQVLEVAEFMSDATRIPLLDDGTGYGNFNSV RRLVRKLEQRGVAGVCVEDKLPKTNFSFVRGGA QPLADAEFAGKIGAAKASQHDPDFVVARTEA LIAGQGLAEALRRAELYRCAGADAILVHSARSDA DEVRAFKAEWGDRLPVVIVPTKYRTPTVEFAED GFSTVVWANHLMRSLRAMQITASTIFAEQHLR NVEGAVAPLAEVFEIQGEDELAEEARWLPRRN TPRR	303	EDKLFPKMNS	unknown
GCF14								
<i>Amycolatopsis xylanica</i>	DSM 45285	GCF_9001 07045.1	NZ_FNONO 1000001.1	45	MDAFSPALRELLDRPGPVRAAGAHNPLGARLAE RAGFDVAVSSGLEISASQGLPDADILTMTELLAV AQSMMAAVSVPIADCCDAGYGNASNMHLVR RYEAGVAACVIEDKTFPKVNSFVPGRTLLPAE DFCNKIAAAKAAQSRPDLVVIARLEALVAGWGL DEALRRGEAYAEAGADMVLIHDKGSKSPQPVLEF LQRWRRPVPVAVVPTTYHTVTADELSDAGAKLV IYANHGLRAAITAITSADFDTILRDGRTTGIEDRISPL STVFDLQGMQRQFIEAERRFVAERGPVEGVA	298	EDKTFPKVNS	unknown
<i>Kitasatospora cheerisanensis</i>	DSM 101999, KCTC 2395	GCF_0006 96185.1	NZ_KK8539 97.1	34	MRAPSRPPAARLALLSGPEPIRLVGAHNALGAR LAERAGFDGIWASGLEVSASHGVPDADILTMSEL LATAQSMARAVEVPVADCDTGYGNAINVMNT VRRYEAGIAGVCIEDKVFPKMNSFVSGRQQLA QLGEFCGKVEAAKAAQEDPDFVIARVEALIANR GMEEAVRRARAYADAGADAILHDKDRSPASIFE FIDRWDFRKLVPVPTTYYSVTVDLAAAGVKM VIYANQGLRASIAAVSRTFEELSAGTSAPVEERIA PLDLVFDLQGVPELKRNEELYLRSDAPVEEGAPH RATPDPAADAAALVASHAN	324	EDKVFPKMNS	unknown
GCF15								
<i>Streptomyces aureocirculatus</i>	DSM 40386, ISP-5386	GCF_0007 20475.1	NZ_JOAP01 000003.1	45	MSQGDLVVRVAGAHDMAGTALQQAQGFQAVW SSSLEVSASRCLPDASVLTMTLEYLAAANMQKAI DIPVVADVDTGYGNLNVAMVHEYEAGITA VAIEDKLYPKVNSFAEVAQTLPLTDFAQKIETAK NAQRGEDFYVIARTEALIDGLGVEEALKRCTAYA DAGADAILHSKKKDEGEIVFLDGDWDRKPVVI VPTTPQWSAAEATKHGVSVIYANQGLRATVQ	274	EDKLYPKVNS	unknown

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
					ALRTTGTIYRDGTSLGVESEIAPVSDVFALQRLQDWLALAE			
<i>Streptomyces silvensis</i>	ATCC 53525	GCF_001482415.1	NZ_LOCL01000042.1	51	MTSHRASFRVMSQDGLVRVAGAHAMGATL AQQAGFQAVWSSSEVSASRCLPDASVLTMT LAAAANMQKAIIDIPVADVDTGYGNLNV MVHEYEAGITAVAMEDKLYPKVNSFAEAAQTL LPLADFAQKIETAKNAQRGEFVIARTALIDGL GVEEALKRCTAYADAGADAILHKKKDEGEIV LDGWDGRKPVVIVPTTYPQWSAAEATKHGVS VIYANQGLRATVQALRTTGTIYRDGTSLGVESEI APVSDVFALQRLQDWLALAE	285	EDKLYPKVNS	unknown
GCF16								
<i>Nonomuraea sp.</i>	G22-1	(N/A)	(N/A)	38	MSVYGQRDRPTAEPAPSPGARLRALLGGSEP AVLMGAHDGLSARVAAEAGFPALWASGLCISTA LGVRDSEASWTELLEMAARVVAASGLPVLVDG DTGHGNFNTARRFTARVEQIGGAGVCLEDKLP KMNSFVGDGHELTGIGFCGKIAACRDALADPD FVIVARTEALIAGAGLDEALRRGEAYRRAGADAL FIHSRQSTVSEIAQFAREWQSRPLVIAPTTYHTT PMEEYVKLGISGVIWANQSMRASLAAMRKACE SLRRDGPAAVEDGISSLEELFSLMRYQELAEDEKR YGDQAPGIAELMATRKPGS	320	EDKLFPMNS	unknown
<i>Nonomuraea candida</i>	DSM 45086, NRRL B-24552	GCF_000725485.1	NZ_JOAG01000015.1	34	MSVYGQRDRPTAEPAPSPGARLRALLGGSEP AVLMGAHDGLSARVAAEAGFPALWASGLCISTA LGVRDSEASWTELLEMAARVVAASGLPVLVDG DTGHGNFNTARRFTARVEQIGGAGVCLEDKLP KMNSFVGDGHELTGIGFCGKIAACRDALADPD FVIVARTEALIAGAGLDEALRRGEAYRRAGADAL FIHSRQSTVSEIAQFAREWQSRPLVIAPTTYHTT PMEEYVKLGISGVIWANQSMRASLAAMRKACE SLRRDGPAAVEDGISSLEELFSLMRYQELAEDEKR YGDQAPGIAELVATRKPGS	320	EDKLFPMNS	2-phosphinomethyl- malic acid + des- methylphosphino- thricin, possibly other H-phos- phinates ⁶
GCF17								
<i>Streptomyces fradiae</i>	HZ1738	(N/A)	EU924263.1 Region on contig edge.	(N/A)	MQRPIVYVGMASDLHPGHINLSRAELGDITIG LLTDAAIASYKRLPHMTYEQKAVVENLKGVASV VPQRTLDYAEHLRTVRPDDVHGDWQTVQQR HTRERVIEWLSEWGGKLEIPYTPGISSTLHSSVK EVGTTNPNVRLSRLRLDSEKIVRILEVHNGLTGLI ENSKVTVDNQAREFDGMWSSSLTDSLARGKPD TEAVDVSSRLQMVNLFVTTKPLVFDGDTGGK PEHFGFTVRSRLERLGVSAVIVEDKEGLKRNLSFGT DVPQTQSSVEDFSARIRIGKRAQITDDFMVIARIE SLILEKGMADAVHRAEAYVDAGADGIMHSRQS DPAEIFEFCRYFDKLPVPLVVPVTSYSSVRESEL ADAGVNMVIYANHLMRVYPQVTKVQVQSLQH GRAHAESMLASIKDALSIIPENS	435	EDKEGLKRN	fosfomycin ¹⁷
<i>Streptomyces wedmorensis</i>	DSM 41676, ATCC 21239, NRRL 426	GCF_000716445.1	NZ_JNWK01000026.1 supercluster	37	MQRPIVYVGMASDLHPGHINLSRAELGDITIG LLTDAAIASYKRLPHMTYEQKAVVENLKGVASV VPQRTLDYAEHLRTVRPDDVHGDWQTVQQR HTRERVIEWLSEWGGKLEIPYTPGISSTLHSSVK EVGTTNPNVRLSRLRLDSEKIVRILEVHNGLTGLI ENSKVTVDNQAREFDGMWSSSLTDSLARGKPD TEAVDVSSRLQMVNLFVTTKPLVFDGDTGGK PEHFGFTVRSRLERLGVSAVIVEDKEGLKRNLSFGT DVPQTQSSVEDFSERIRIGKRAQITDDFMVIARIE SLILEKGMADAVHRAEAYVDAGADGIMHSRQS DPAEIFEFCRYFDKLPVPLVVPVTSYSSVRESEL	435	EDKEGLKRN	fosfomycin ^{18,19}

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
					ADAGVNMVIYANHLMRVYPOVTKVQVQILQH GRAHEAESMLASIKDALSIIPENAG			
Singletons								
<i>Actinoalloteichus cyanogriseus</i>	DSM 43889	GCF_000429185.2	NZ_AUBJ02000001.1	22	MIREKELTYLMEAHDLGSLARIAEVEGFOAIWASG LSMSTALGVRDSEASWSQLLGVVEAMVEATT VPIVVDGDTGYGNFNTARRFVAKAERLGAVGVC LEDKIFPKMNSFVGDSTLAEVSDFTAKIRACKDS QRSSDFVLVARVEALIAGRGLPEALDRAHAYHEA GADAFIHSRRSDATEILDFCREWGERLPLVIAPTT YASTPSSEFQAAGVSAVIWANHSMRAAVSAMR RVCREFAKQSVHRTESELASLAEVFELMEYSEID EAERRAYRV	280	EDKIFPKMNS	unknown
<i>Actinokineospora auranticolor</i>	DSM 44650	GCF_041897805.1	NZ_CP154825.1	35	MTEDTRGQVRQVETRAAALREAFEGRLLRVAG AHDGLSARLVAEAGFDVAVWASGLEISAHGLPD ISLLGMAEFLLAAATMQSATAVPVVADCDTGF GDRNAFTMMRYENVGIAVCIEDKVPKLNFS LGAGQELLATEDFGAKLRAAKQAQHDPTVLIA RTEAFICGYGVVEALARCHHYVDGADAVLVHS KAADSSEVVSFTRRWQRRAPVVVPTTYTAFSV AEAQEGVAMVIYANQGMRAVRAVDAWA AVLADGSTVGIESTIASVKDIFELSGMSQWLGRG	295	EDKVFPKLNS	unknown
<i>Actinopolyspora mزابensis</i>	DSM 45460	GCF_900101095.1	NZ_FNFM01000005.1	21	MFLNHYYVLETVLSISTRTEPESARQPEAGSGR PGRFRRLASPELSFAMEAHDGLSARIAQAEF RALWASGLSISTAMGLRDNNEASWSQFLQVVEL MADATSPVIVDGDSDGHGNSARRFFRKAQEQ VGAAAVSLEDKIFPKMNSFVGTHELAPAEFCA KIRACKDSQDPEFCIARTESLIAGVGMREAIOR AEQYRLAGADAFIHSKKQVSDIEVFSQEWGER LPLVIAPTTTAAATPTDIFRRGISTVIWANHSMR AAFAAIRQTCREIRDTSVSHLETAPLSNVFDLL DYDELAERESRFFTDHPMNG	325	EDKIFPKMNS	unknown
<i>Bacillus spizizeni</i>	ATCC 6633, NRS-231	GCF_000177595.1	NZ_ADGS01000025.1	11	MKAKKRLRELLYSNQVVRVMGAHNGLSAKLAEQ AGFHAIWASGLEISASYPDPANILMTENLQAA VVMNESTSPIICDCDSGYGVNNVIRVMKEYER NGIAGICIEDKQFPKLNSFVKGSKQLADIDFSNKI RAAKDVQKNPDFVVIARIEALIAQGGMDEALNR AYAYEAAGADAILHSEKQNPNEIKFVKQFTGA VPIVIVPTTYPHITVKEMELLGINMVIYANHGLRS SIKAMOETFSQILLDGNVTGVEDNIVSMKTVFEL QGMYDMRKQEDMYNSGTSVISTIK	296	EDKQFPKLNS	rhizocitins ²⁰
<i>Bacillus velezensis</i>	AA3, NRRL B-41580	GCF_001461825.1	NZ_LLZC01000022.1	19	MVKNRKSIFRDALDSKSLVKVAGAHDLGSLAKLA EKNGFNAVWASGLGISAVQTPDASILMTFELE AAVIMNESCNPVIADCDSDGYGNIHNVTRMIKK YEAAGIAGVCIEDKVPKLNSFDDRRQILVSTEEF CAKIRAAKMAQQNDDFVLARVEALIAKLGQEEA YTRAKAYVHAGADAILHSEKQSPDEIEFVNNW DVDAPLVVPTKYPTLSMEQLEKLGKVSIAANQ ALRASVKAINDTFESIINNKSLLQIENDIVSVNEIF DIQDVPGMKQLERLIHQRPAN	295	EDKVYPKLNS	phosphonoalamide E + F ²¹
<i>Glycomyces</i> sp.	NRRL B-16210	GCF_000719515.1	NZ_JOGR01000003.1 Region on contig edge.	11	MSADLIHPGHINILNRAELGDVTIGLLTDAAIAS YKRLPHMTFEQRAKAVVANLKGTVAVIPQETLDY VPLNRSVKPDFVHGGDDWQTGVQRETRQVRV DALAEWGGELVEVPYQTQGISSTQLNASVKEVGT TPDVRLTRLRLIDAKPIVRVLEAHSGLTGLIETA QAERGGKLVFEDAMWSSSLTDSARGKPDIELV DLNSRLQTINDLFEVTTKPLIYDGDGTGGKPEHFSY TVRSRLRLGVSAVIEDKEGLKRNLSFGTEVAQTQ STIEDFVHRIQVGKKAQVTDDFMIARIESLILEM GMEDAVTRAKAYIEGGADGIMHSRQKSPDEIFE FCERFAAFETKVLVVVPTSYNQVTEEEFIERGVN	426	EDKEGLKRNNS	phosphonoglycans (2-HEP containing polysaccharides) ²²

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
					VVIYANQLMRASYKAMSGVAKSILENARSAEVD SQIANIKEALAIIPENES			
<i>Kibdelosporangium banguiense</i>	DSM 46670	GCF_017876405.1	NZ_JAGINW01000001.1 supercluster	47	MTVDLSNKKVTHSPGSRLLRILESPGVSTFLGVH DALSAIAADCGYPALWASGLGMSALGLRDCD EASWTELLDITARMVEATNLPILVDGDTGYGNF NTARRFAMRAERAGAAGVCIEDKVFPKMNSFV GDGHALAPIPEFCGKIAACKDAVRDSSFVLRV EALAGAGMGVALERAEAYRAAGADAFIHSRM PTVAQIAEFTLGWNDKPLIAPITTYHRTPIAEFER LGISGVIWANQAMRAAVALVAVKRVCAALHDGPA GIDSSISLPEVFSLLDYDALERDEQLYGSYEHRRNP L	304	EDKVFPKMNS	unknown
<i>Kitasatospora phosalacinea</i>	DSM 43860, NRRL B-16230	GCF_000716545.1	NZ_JNWZ01000012.1 supercluster	24	MTSPTSLPDGGTAPSTPGQRLRHLLAQTEPVQL MGAHDGLSARIAAEFGPALWASGLCMSTARG VRDSDEATWSELDDLAATMIEAAPGVPLIDGD TGYGNFNTARRFAARAERIGAAGVCFEDKVFPK MNSFFGDGHALAPVPEFCGKIRACKDGGQDPG FLVARTETLIAGRPEEALERAEAYAEAGDALF IHSRKPTVEIAGFLERYDGRPLVPIPTTFTHTPTV DEFGR LGVSGVIWANHSMRASFAAMRDVCCQI RSNRGISAVEGQVSLKEVFGLLYEALAEAEAY TGALGGAAGV	312	EDKVFPKMNS	phosalacine ^{23,24}
<i>Lentzea</i> sp.	TUE H45	(N/A)	(N/A) supercluster	35	MTKAELRELTRPAVSRIVGARDPLTARMVEEA GFDGVVWSSFELSATRALPDGLTMTCELDAA ALIDGATVLPVSLVDCDTGFGTAINLVRVRLCEAA GIAGICVEDKVFPKNSYLDGGQVLEDPDAFAQ RVEAAVRSRRDQDFTTVARCEALAGAGMDEA VRRHHYVDAGADAVLVHKKRREPDEIVEFLHR WRRRAPVVPVPTTYRWLSAEAQDAGVSLVIYA NQALRASVLAVRTLSEIHLRGDAATAEDSIAPV KEVFALTRTAEWEEWGA	284	EDKVFPKRNS	unknown
<i>Nocardia mexicana</i>	DSM 44952	GCF_003350525.1	NZ_QQAZ01000001.1	38	MTHTNGHGSKAQLRKLKLAGDRLARVVGVGDG LSALLASRHGFDALWGSGLSIAHALPDASILT MTEFLQATAVIDAATPLPTIADCDTGFGDNNV MRATREYERRGIAIGICMEDKQFPKRNSFSGGQQ LAPIAEFAQKIRAAKSVQVSPDFVIARVESLIAET GMADALERGARYAQAGADALLHSKSESPDEV EFAAEWNRQSDPLPLVAVPTTYYSASVDELERG GFSMVIYANQSLRAVVRVNDVFARLSSAATA EMEEQLIPVVEVLIDIGMDSLLTSDVDG	295	EDKQFPKRNS	unknown
<i>Nocardia pseudobrasiliensis</i>	DSM 44290	GCF_003350585.1	NZ_QQBC01000006.1	54	MRENLPVTRPPAAGVAGAQRLGLAEPEPSYL MGAHDGLSARIAAEAGFPALWASGLCMSTILGC RDNDVSWSELDDLTARMVEAGGVPLVDADT GYGNFNTARRFAARAQRIGAAGICVEDKVFPKM NSFFGEQHPAPIGEFCAKIAACRDCVDEDFVIVA RVEALAGAGMAEALRRAEAYREAGADAFIHSR QRTVEEIAEFTREWGARLPLVIAPITTYTVPQATF AELGIAAIVIANHAMRASVAAMREACRALRRD GAAAVEPVITPLDELFSLMGYPELEADQAHYSSL AQKWVDS	308	EDKVFPKMNS	unknown
<i>Nocardia tenerifensis</i> (1)	DSM 44704(-1), NBRC 101015	GCF_003202065.1	NZ_QKFIF01000021.1	47	MSENFPGRTGATAGRLRLGLADPQPSYLMGA HDGLSARIAAEAGFTGVWASGLCMSTVLGARDS DEISWAEELDLVARIVEGGGLPVLDADTGYGNF NTARRFAARAERIGAAGMCLEDKVFPKMNSYFG DSHPLAPVGDIMCAKLAACRDQVDAEFVLVART EALAGAGLAEALRRAEAYRQAGADAFIHSRRRT VDEIAEFTAEWGARLPLVIAPITTYTVPRTFAEL GIAAIVIANQSMRAAVALVAVKRVCAALHDGPA AAVEPGIASLRELFDMGYPELEADEAHYTLAQ KWSDAKPVVRVQSHSMSDEDVKHVS	323	EDKVFPKMNS	unknown

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
<i>Nocardia tenerifensis</i> (2)	DSM 44704(-2), NBRC 101015	GCF_0032 02065.1	NZ_QKFIF0 1000015.1 supercluster	47	MEKVRALASLIFERAGLDFLMGAHDAVSAQIAQ DAGFPGIWWVSGLSATNGLRDSNELSWTQVM ERIEMLSDRIAPALVDMMDTGYGDFNNVRLAVR RLRRIGAGGACIEDKLPKTNFLGDGQALAEFG EFCGRLLKAAKDTAADVFLVARCEALVAGRPLAEA LDRCGMYAESGADAVLIHSSKKNPDEILAFMAE WDGSAVAVVPTKYADVPASVLEEAGVSVAIW ANQSLRAAIAAMRRLCTQLAQRTMRGLEGEIA ELSQQVFELANNAELDQAKTRYRYVPPSREAVAN NGARTRAAETVDDVLRLEAWSAVQSTEDPAET LLAHVRDTPFPYQIGTQELSDLPVVDRTAYEND VAEFTSAASTGKYCLTSSGTTGNPLTVLDDASW YAVNYHFTQICDLAGLPPDGFAGELAVLFVSN KPGRDTFVRPLPSLNYGLYARIQLDLAKSTRYS RLGAEILYGKPTYLLDLRAALIAQGFATPPWSRL VLVSGEPLHADDRARLTDYYPAPVVDALASTEG GLIAATRPGEETYQVFPENVRLEVLDDGVVRDS GVGELVLTNLVYRDTVFLRYRTGDRAELETDAAG SQRLRRLWGREPRTLSFGDRRLPTRDLTERFGSL PGMGDFQIVSRDLGALLRWMPDAGYQEPETLR RSLRAAVDELLPDQDVEFELCSRIPTPGGKRRFR	704	EDKLFPKTNS	unknown
<i>Pantoea ananatis</i> (2)	LMG 5342(-4)	GCF_0002 83875.1	NC_016816 .1	9	MQKKIDRLGSVNSKNRILREMISSLSFLMEAH NGLSAIAQNSGFSGLWASGLTISASLGLSDRNE ASWTQVLDVAEFMADHVDIPILLDGDGTGFSYL NVMRLVKKLCQKGISGVCLDQVFPKMNSFIGEK QELIAIEDFCSKIAAKDTQLDDDFVLVARTEALIS GRGMAEALRAEAYHEAGADAILHSSKTDASEI VEFSNEWAERSPLVIVPTKYSTPTELYRDLKISTV IWANHSLRASVVAIENTKKIFETQSIKEVEQDIVS LQDLFLTNEVNSAIAEKYGL	299	EDKVFPKMNS	unkown
<i>Pseudomonas syringae</i>	PB-5123	(N/A)	JX102649 Region on contig edge.	(N/A)	MSSITHAEQFRRLFNGLDLILEAHNGLSARIVE EAGFSIAIWASGLISLALGRDCNEASWSQVVEV TQSIHDAVTIPILFDGDSGFGNFNNVRHIVKRLSH YGIAGISLEDKLPFKMNSFITGAHALSPVEEFCGI KAAQDSKCDRHFTVIARTALIALGLGVEAALERA QHYHDAGADGIFHISKTDGLDILEFGRRWERRS PLVVAPTTYINTPLALLEEVGVSIVICANHLMRASI QAMRKAAGQIRKDNSISTINSIDISLTFVFSLLNY DELQAAERLYAR	291	EDKLFPKMNS	fosfomycin ²⁵
<i>Saccharopolyspora spinosa</i>	DSM 44228, NRRL 18395	GCF_0001 94155.1	NZ_GL8778 79.1	38	MLDSPMVFLMEAHDLGSLARIAQSEGFDALWA SGFSISTSLGLRDSDEASWSQLLSVVEYMVAGTS VPIVVDGDTGYGNFNTARRFLVSAERQGAAGVC FEDKVPFKMNSFVGDSHRLADIDEFAAKIAACSE ARNDEDFCIARTALIAIRGVDEALKRAEAYRKA GATAIFVHSRRQVADEIEQFAAAWADRPLVIA PTTYASTSADTFRELGISAVIWANHSMRAAVTA MRDVCRSIKASESVLSAEAKIASLKEIFFMDYAEI EETERRLLRGTSAAD	287	EDKVFPKMNS	unknown
<i>Stackebrandtia nassauensis</i>	DSM 44728, NRRL B-16338	GCF_0000 24545.1	NC_013947 .1	18	MSSKPTVYVGMADLVHPGHINILQRAELGDV TIGLLTDAAIASVXRPLPHMTYEQRKAVVENIKGV AAVVPQETLDYVENLEKLPNFVHGDWKTG VQQQTRQRVIDALAQWNGELVEVGYTEGISSTQ LNEVKEVGTTPNVRLRLRLIENKPIVRIMESH SPLTGLIETTAEHNKRKVEFDGMWSSSLDST ARGKPDIELVDPASRMQGINDLFDVTTKPLIYDG DTGGKPEHFSYTVRSLRLGVSIIIEDKEGLKRNS LFGTGAQQTQCSIEDMVHKIQVGKKAQVTKDF MVIARIESLILEQGMDDAVKRARAYIDEGGVVGI MIHSRQKTPDEVFEFCFLQKFKRVPLVVVPTS YNTVVEDEFAKRGVNVVYANQLMRASYKAMA SVATSILENGRSAEVDSQIANIKEALAIIPENLA	437	EDKEGLKRNS	phosphonoglycans ²²

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ³ NS)	phosphonate product
<i>Streptomyces durhamensis</i>	DSM 40539, NRRL B-3309	GCF_000725475.1	NZ_JNXR01000046.1 Region on contig edge.	43	MLKRSYPSFRSLVEKGLVVRVAGAHDALGAVLAEEQAGFDVWSSSEVSAAARCLPDASVLTMTTEYLDAAANMQKALGIPVIADCDTGYGNLNNLVHVMVHEYEAAGITAVCMEDKLFKLSFASGQDQTLSTEFCSKIRAAKGAQATGDFVVARTEALINGLSVDVALERSHAYCDAGADAVLIHKSXTKDQVEEFLSRWDGRRPVVIVPTTYPDWHVDIAKAGVSVVIYANQGLRATVASLRETTFESICRHGDSTDLEDRIAAVSDVFLQKLKEWQKLDA	286	EDKLFPPKLNS	valinophos ^{6,26}
<i>Streptomyces glauciniger</i>	DSM 41867, CGMCC 4.1858	GCF_900188405.1	NZ_FZOF01000041.1 Region on contig edge. supercluster	35	MTSPQLTAPTMPRPKPGTGAALKRALFMRPGITRIVGAHNPLGARLAERAGFDGVWSSGLEISASQGLPDTILMTTELHTVAASLAAVDIPVVADCDAGYGNFVNMHMIRRYEASGIAAVSIEDKLFKPVNSFIPRRQKLASIEEFSGLRAAKSVQEDPDFMVIARIEALAGWDMEEALRRGEAYAAAGADAVLIHAKGSEAEPLFLSRWQDLPVWVPTTYHTVTADLSAAGAKMVIYANQGLRAGISAVSETFTILRDGRTTALIEDRIAPMKTVDLQGMQVMADEARFLPNSGKTDDQ	312	EDKLFPPKVNS	unknown
<i>Streptomyces iranensis</i>	DSM 41954	GCF_042466515.1	NZ_CP136563.1 supercluster	70	MANGNLIRAAGAHDALGSALAQQAGFQAVWVASSEVSASRCLPDASVLTMTTEVLAANMQKAVDIPVVADVDGTGFGNNLNVAHMVREYEAAGITAVCIEDKVYPKVNSFAATDHELLPVGTFTRKLATAKAAQDQEDLYVIARTEALINNKGVDEALDRCYAYAEAGADAVLVHKKKDDQGEIVEFLDSWDGRSPVIVPTTYPQWASDAEKHGVSMMVIYANQGLRATIQALRDAFRITYDDGTSLGVENAIAPVSDIFALQKLLDWLALAE	274	EDKVYPKVNS	unknown
<i>Streptomyces lavendulae</i>	Fujisawa #8006	GCF_000715625.1	NZ_JNXL01000002.1 supercluster	26	MTAAATRASEGITDGTSLRDLFERPGVVRAGAHNPLGARLAERAGFDGVWSSGLEVSASQGVDPDTILMTSELLAVAGSLASAVSPVADCDAGYGNAHNVMMNIRRYEAGISAVSIEDKRFKPVNSFIPGRQELAPIGEFCGKLAAAKAAQSGSELMVIARIEALIWAGWMDALLRGEAYADAGADAVLIHAKGSSPDPIELFLNRWRLPVVPTTYHTITAAELGEAGAKMVIYANHGLRAGITAVSRAFEALREGRTTGIEEQIAPLATVFDLQGMPPQKQHEKLYITPYGTSPRAFIMVPGEQQTGLDQRADVLVCQTALERSGVEQVAVYAADQPPTRELQDQDVVAVGVGTDAAAWVLSTPANYPGSTLVLPADVLEAGPLRQLASNESDVAVLVDVSTRSGAARRPDVAVGVSLSSSI RGGRRLTAGSSSLVTGFGGSEVEAEFTGAAVFSARGFAALVDAAEKRRAGSTATVVELLADVLLGGLQVHAIEVASGWTELRADDLRYGGEAMATGGAE R	534	EDKIFPKRNS	fosmidomycin ^{27,28}
<i>Streptomyces mutomicini</i>	DSM 41691, NRRL B-65393	GCF_001700505.1	NZ_MAPV01000141.1 Region on contig edge.	54	MTTSSARLELLTAPQAAARAAGHDGLTARLVEEAGFDVWVASSFETASRALPDMSLLTMTDYLQAAASWMVQSTSLPVLADCDTGFGRNLNIATYVRQYEAAGVAGICLEDKVFPKNSFLAVGQTLDPAEFAARIRVAKQAQHNPEFVVARVEALIAGTGMADALARAHQYADAGADAILIHSKSQEPHEIQEFLQWQRRTPVWVPTTYFRWKTEASRAGASLVIYANQGLRAAVQAVRDVLEIRLTGDSSTSSDVIA PVKEIFRLTDVDTWNALES	285	EDKVFPKKNNS	unknown
<i>Streptomyces phyllanthi</i>	DSM 117319, TISTR 2346	GCF_009377205.1	NZ_OX419519.1 supercluster	45	MTVEGPGTPVPRGPAPSQPADRLRELLTSDSLGFLMEAHSGLSAKIVQDEGFDIAIWASGLSIATAFGVRDSELSWTQVQDVVGSITEVSTPLVMDGDTGFGNFNNARRLVTSLCRMGVAGVCIEDKVFPKLSFVGERHPLAEIDFCGKIKACKDAQTVPAFTVVARVEALVAGHGFGEALRRAEAYREAGADAVFIHKKTDGKEILRFAQEWAEACPLIVPTTYHTVPTDAFQEHGVSIAVIANHMMRASIAAMRSACRE	314	EDKVFPKLNS	unknown

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
					VRQTRTVTGVERRIAPLKEVFDLLDYPELERASHR YLPQRMPLGEEVW			
<i>Streptomyces rubellomurinus</i>	ATCC 31215	GCF_0009 61885.1	NZ_JZKH01 000051.1 Region on contig edge. supercluster	59	MSIAAGVHDGLSARIAQDAGFDVLWASGLGISA AHAVPDDSLTMTFLEAARVMNDSTDLPLVAD CDTGFGERNVARMVERERAGIAGVCIEDKVF PKRNSLGDGAQDQETIEGFAAKLAAAKKAQQT DFVVVARIETFIAGGVKEDAIARADAYVAAGADA ILHKSRRDGLDFGRSQRKDVPLVAVPTTYP AVTADDLHEAGFSLAIYANQALRASITSMRESLR QITHDRSSLRIEPPKAKLSEVFELQR	261	EDKVFPKRNS	FR900098 ^{28,29}
<i>Streptomyces seoulensis</i>	DSM 41840, KCTC 9819	GCF_0043 28625.1	NZ_CP0322 29.1 supercluster	23	MSAATTEASPVSAARTADRLRDLFARPGVVRIA GAHNPLGARLAERAGFDGVVSSGLEVSASQGV PDTDLTMSSELLGVASSMAAADIPVVADCDAG YGNAHNVMMNIRRYEAGAAVSIEDKRFPKVN SFIPGRQELAPIGEFCKLAAAKEAQRHPLMVI ARIEALIAWGMDALRRGEAYARAGADAVLIH AKGASPEPILQFLREWRLPTPVVVPTTYTITAT ELGEAGAKLVIYANHGLRAGITAVSDTFQAILRD DRTTGVEDRIAPLATVFDLQGMMAQKRHEAEFI TPFESRARAADVPAEEPSGRAADLLDHQTAAL RRAGIESVSLVTSGEVPAQLPQDTAVLAGHTDAA GAVLELPATQLSATLLSSDAYVQSEPLARLLAAG GDVTLVDVSARGKHRADAVPLRLDSRVHEGRR LSAGDGIRTVGVGGQADGEFAGAAVFPQGF AALREAAGKRRANGSPATLADLLDVEGGHQV GAVEIGSGWMLRTAADAHAAGLLTGQGEVR	532	EDKRFPKVNS	unknown
<i>Streptomyces</i> sp.	NRRL F-525	GCF_0007 16605.1	NZ_JNXX01 000019.1	43	MSPVKSRLALLHDADLAKAAGAHNPLTAQLVEQ AGFDVVWASGLEIAASLGVPDANILSMNECLAV ARAMVEKVSVPVLADCDSDGFGGIGNVVMHVR YEAAGVQGVCIEDKTFPKLNSFVAGNQALVPID FAGKIAAATVRSDFVIAIEAFISGYGLDEAL RRAEAYELAGADALLHKSRTTPDEIYFCAAYDG QLPIVVVPTTYSVTMEELRAGGASLAIYANQGL RGAITSIRQIFGSILAEGTAAPVENQLAPLGDVFG YQDVAALLSAEDRFESIGQGVAKQLNGAREERR NADVVER	313	EDKTFPKLNS	O-phosphonoacetic acid serine ³
<i>Thermomono- spora echinospora</i>	DSM 43163	GCF_9001 08175.1	NZ_FNVO0 1000023.1	27	MPMSLPEIRALPALLRPPRNPVLVAGAHGDMT ARLAQRNGFGAVWASGFSLATSHAVPDASVLS MSESLAAVRMMRAATDLPIADCDTGFEEDTADI TSLVQSYEAAGVSACIEDKAFPKYNSFLSAPQTL ETTSVFSKIEAAKKAQRGTGFMVAARTEAFIAG LGARQAIARAEAYVAGADMILVHASKASPHEIY EFMELWDHRSPPVAVIPTSYHGVITDELGSAGIA MVIYAHAMRAAARAVNMALRSIIDAGTTTHIE KDLATLQEVFEIQGATGLRGSRSAGGEAGSRTRN ASKNFISPGDGTGRDVT	318	EDKAFPKYNS	unknown
<i>Umezawaea tangerina</i>	DSM 44720	GCF_0030 02815.1	NZ_PVTF01 000007.1 supercluster	43	MLRREHQSFRVLAANKVVRLAGAHDMGAAL AEQAGFGQVWASSLEMSAARCLPDASVMTMT EYLEGASNMQKALSIPVADCDTGYNLNVVA HMYHEYEAAGITAVCMEDKLFPMKNSFAGGAQ TLLSTEAFASKVETAKNAQRDPDFVIARTALIS GLSVEEALERCHAYADAGADAVLIHSAKAKVNDQ VLEFLRAWNGHRPVPVVPTTYPEWHVDDAAAA GVSVVIYANQGLRATVSALRDTFKTILGETTSLE SRIASVTEVFELQNLDDWQKLEV	286	EDKLFPKMNS	unknown
Total no. of strains: 72 (with 74 BGCs / 74 pepM genes with EDKX ₅ NS consensus motif)		Ø No. of PBGCs: 36.3			Ø No. of PepM aa: 316		DSMZ/Tue strains: 54 No. of additionally included non- DSMZ/Tue strains: 18	

Strain	strain designations	Genome accession	P-BGC accession	total no. BGCs	PepM sequence	PepM aa	PepM consensus motif (EDKX ⁵ NS)	phosphonate product
							And unknown: 2 (<i>S. silvensis</i> and <i>S. sp.</i> 31A4)	
45 not previously reported Tue/DSM strains identified as phosphonate producers (17 BGCs from 16 strains in singletons + 8 BGCs from 8 strains in GCFs with no known producers (á 5 GCFs, since GCF09 + GCF15 contain non-TUE/DSM strains, that are not known producers) + 21 BGCs from 21 strains in GCFs with known producers)								
25 known PNP producers (16 non DSMZ/TUE strains (17 BGCs due to <i>P. ananatis</i> harbouring 2 BGCs) + 9 DSM/Tue strains)								
2 non DSMZ/TUE unknown producer strains, included for close homology with strains of interest (<i>S. silvensis</i> and <i>S. sp.</i> 31A4)								

Table S2. Bioassay results in the test of putative phosphonate producers against *Escherichia coli* WM6242, *E. coli* K12 and *Kocuria rhizophila*.

Inhibition zones were documented by measurement of diameter including the 8 mm diameter agar-block of grown actinomycetes when inhibition of test strains was observed. Values are in [mm]. Strain did not grow on the selected cultivation media (N/A) or was not measured (N/A). Plus (+) indicates presence of putative phosphonate peaks in ³¹P NMR, minus (-) absence under any tested condition. Strain names and designations highlighted in bold lettering do not belong to any GCF that includes sequences from known producers at a cutoff value of 0.612 in the BiG-SCAPE analysis.

Strain	DSMZ	other strain designations	³¹ P NMR measured	Inhibition zones in the test against <i>E. coli</i> WM 6242										Inhibition zones in the test against <i>E. coli</i> K12										Inhibition zones in the test against <i>Kocuria rhizophila</i>											
				Media	HM	OM	R5	SFM	NL200	NL300	NL400	NL410	NL500	NL800	HM	OM	R5	SFM	NL200	NL300	NL400	NL410	NL500	NL800	HM	OM	R5	SFM	NL200	NL300	NL400	NL410	NL500	NL800	
<i>Actinoalloteichus cyanogriseus</i>	43889		N/A	0	0	0	0	0	N/A	N/A	0	0	0	0	0	0	0	0	N/A	N/A	0	0	0	0	20	26	26	26	N/A	N/A	26	26	26	26	
<i>Actinokineospora auranticolor</i>	44650		N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	14	16	15	11	12	13	16	0		
<i>Actinopolyspora mزابensis</i>	45460		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A		
<i>Amycolatopsis xylanica</i>	45285		N/A	0	0	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	12,5	N/A	0	0	9	12	17	0		
<i>Goodfellowiella coeruleoviolacea</i>	43935		N/A	0	0	N/A	13	0	0	15	13	0	13	0	0	N/A	13,5	0	10	14	12	0	11	10	14	23	N/A	0	18	23	18	0	18		
<i>Kibdelosporangium banguiense</i>	46670		+	0	N/A	N/A	0	0	0	9	0	0	0	0	0	N/A	10	0	9	0	0	0	9	9,5	13	N/A	18	N/A	18	15	10	10	11		
<i>Kitasatospora cheerisanensis</i>	101999	KCTC 2395	+	0	0	0	0	0	N/A	0	0	N/A	0	0	0	0	0	N/A	0	0	N/A	0	0	0	0	0	0	0	0	N/A	0	0	N/A	11	
<i>Kitasatospora fedleri</i>	114396	TU 4103	+	0	N/A	0	0	0	N/A	0	0	N/A	0	0	0	0	0	N/A	N/A	N/A	0	N/A	0	0	0	0	9	0	0	N/A	0	10	N/A	9	
<i>Saccharopolyspora spinosa</i>	44228	NRRL 18395	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
<i>Streptomyces glauciniger</i>	41867	CGMCC 4.1858	+	0	0	16	0	0	N/A	0	0	N/A	0	0	10	0	0	N/A	0	0	N/A	0	9,5	0	13	0	0	N/A	12	12	N/A	0	0		
<i>Streptomyces iranensis</i>	41954		+	9,5	N/A	N/A	0	0	N/A	N/A	12	N/A	N/A	11	N/A	N/A	0	0	N/A	N/A	13,5	N/A	N/A	11	N/A	N/A	10	0	N/A	13	N/A	N/A	11,5		
<i>Streptomyces mutomycini</i>	41691	NRRL B-65393	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	9,5	0	0	9	8,5	9	13	8,5	0			
<i>Streptomyces rimosus</i>	40260	ATCC 10970	N/A	15	11	N/A	9	11	N/A	10	10	N/A	14	10,5	13,5	0	10,5	12	N/A	14	10,5	N/A	14	10	11	0	13	9	N/A	12	10,5	N/A	16		
<i>Streptomyces seoulensis</i>	41840	KCTC 9819	+	0	0	0	0	0	N/A	0	0	N/A	0	0	0	0	0	N/A	0	0	N/A	0	0	0	0	0	0	0	0	0	0	N/A	0		
<i>Thermomonospora echinospora</i>	43163		N/A	N/A	0	N/A	0	N/A	N/A	N/A	0	0	0	N/A	0	N/A	0	N/A	N/A	N/A	0	0	11	0	13	0	0	0	11	11	0	13	0		
<i>Umezawaea tangerina</i>	44720		N/A	0	0	0	0	0	0	0	0	0	0	0	0	N/A	0	0	0	0	0	0	0	0	0	18	N/A	0	0	0	0	0	0		
<i>Kitasatospora atroaurantiaca</i>	41649		+	0	0	0	0	9,5	N/A	0	10	N/A	0	0	0	0	0	N/A	0	0	N/A	0	9	0	0	0	9	N/A	10	0	0	N/A	9		
<i>Kitasatospora phosalacinea</i>	43860	NRRL B-16230	N/A	0	0	18	0	14	N/A	0	0	N/A	0	0	0	0	0	N/A	0	0	N/A	0	0	0	0	0	0	0	0	0	0	N/A	0		
<i>Kitasatospora purpeofusca</i>	40283	NRRL B-1817	+	0	0	0	10	0	N/A	11	0	N/A	0	0	0	0	0	N/A	0	0	N/A	0	9	0	0	9	0	0	N/A	10,5	0	N/A	10		
<i>Kitasatospora setae</i>	43861	KM-6054	+	0	0	0	0	11	N/A	0	0	N/A	0	0	0	0	0	N/A	0	0	N/A	0	0	0	10	0	9	N/A	0	0	0	N/A	10		
<i>Salinispora pacifica</i>	45543	CNS-863	N/A	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	10	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A		
<i>Streptomyces alboniger</i>	40043	ATCC 12461	-	0	0	0	0	0	N/A	0	0	N/A	0	0	12	0	0	N/A	0	0	N/A	0	0	9,5	10,5	9,5	9	N/A	0	10	N/A	9,5	0		
<i>Streptomyces bikiniensis</i>	40581	ISP-5580	+	0	9	9	9	11	N/A	0	0	N/A	0	9	11	9,5	0	12	N/A	9	0	N/A	0	9	12,5	12,5	10,5	13	N/A	13	10,5	N/A	10		
<i>Streptomyces chrestomyceticus</i>	40545	NBRC 13444	N/A	11,5	0	9	9	0	N/A	9	0	N/A	9	9	0	0	0	N/A	9	0	N/A	8,5	10	0	9	9,5	0	N/A	10,5	9	N/A	10	0		
<i>Streptomyces fradiae</i>	40943		N/A	14	9,5	0	9	13	N/A	9	10,5	N/A	10,5	0	0	0	0	N/A	9	0	N/A	0	0	0	0	0	0	0	N/A	9	11	N/A	0		
<i>Streptomyces hokutonensis</i>	102214	R1-NS-10	-	0	0	0	9,5	0	11,5	0	0	0	0	0	0	0	0	0	0	0	0	0	8,5	0	10,5	0	0	0	0	0	0	0	0		
<i>Streptomyces monomycini</i>	41801	NRRL B-24309	+	0	12	N/A	12	15	13,5	0	13	13	9	0	9,5	0	10	0	0	8,5	0	0	10	9	10	10	10,5	9	10	15	11	9	0		
<i>Streptomyces regensis</i>	40551	NRRL B-11479	N/A	0	0	11	0	0	N/A	0	0	N/A	0	0	9	0	0	N/A	0	0	N/A	0	15,5	14,5	18	18	14,5	N/A	18	15	N/A	15,5	0		
<i>Streptomyces resistomycificus</i>	40133		+	0	0	0	0	0	N/A	0	0	N/A	0	0	0	0	0	N/A	0	0	N/A	0	9	0	9,5	0	0	N/A	0	0	0	N/A	10		
<i>Streptomyces</i> sp.		A4-2	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	0	0	0	0	0	0	0	0	0	0	0		
<i>Streptomyces</i> sp.		I6	+	0	N/A	10	13	N/A	N/A	N/A	13,5	N/A	0	0	N/A	0	13	N/A	N/A	N/A	0	N/A	0	10,5	N/A	0	18	N/A	N/A	N/A	19	N/A	0		
<i>Streptomyces</i> sp.		TUE 18	+	0	9,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10,5	9	0	8,5	0	10,5	10	10,5	9	12	0			
<i>Streptomyces</i> sp.		TUE 21470	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10	10	0	9	0	10,5	13	10	10	13			
<i>Streptomyces</i> sp.		TUE 3678	+	0	0	N/A	0	0	0	0	0	0	N/A	0	0	N/A	0	0	0	0	0	N/A	N/A	8,5	0	0	N/A	0	0	8,5	0	N/A	N/A		
<i>Streptomyces</i> sp.		TUE 3997	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	13	12	11	0	11	9	10	8,5	14	0			
<i>Streptomyces viridochromogenes</i>	40736	TUE 494	+	0	0	14	0	12	N/A	9	0	N/A	0	0	0	14	0	0	N/A	0	0	N/A	0	0	0	0	0	0	0	0	0	N/A	0		
Total tested				36																															

Table S3. Plasmids used in this study

Name	Description	Source
pIJ10257	<i>Streptomyces</i> expression vector; <i>ermEp*</i> , <i>hygB</i> , <i>phiBT1-int</i> , <i>phiBT1-attP</i> , <i>RK2-oriT</i> , <i>ColE1-ori</i>	JIC StrepStrains; Hong et al., 2005 ³⁰
pRM4	<i>Streptomyces</i> expression vector; <i>ermEp*</i> , <i>aac(3)IV</i> , <i>phiC31-int</i> , <i>phiC31-attP</i> , <i>RK2-oriT</i> , <i>ColE1-ori</i>	University of Tübingen; Menges et al., 2007 ³¹
pGus21	Knock out vector, non-replicative in <i>Streptomyces</i> ; <i>aac(3)IV</i> , <i>RK2-oriT</i> , <i>ermEp*::gusA</i> , <i>pMB1-ori</i> , <i>I-SceI-site</i>	University of Tübingen; Ladwig et al., 2015 ³²
pTC192-km	Source of kanamycin resistance gene <i>neo</i> <i>ColE1-ori</i> , <i>bla</i> , <i>neo</i>	University of Leon; Rodríguez-García et al., 2006 ³³
pBluescript II KS(+)	General <i>E. coli</i> cloning vector; <i>ColE1-ori</i> , <i>bla</i>	Alting-Mees and Short, 1989 ³⁴
pDS0101	pIJ10257 derivative, <i>ermEp*::kfp25</i> ; <i>luxR</i> overexpression	This work
pDS0102	pRM4 derivative, <i>ermEp*::kfp25</i> ; <i>luxR</i> overexpression	This work
pDS0105	pRM4 derivative, <i>ermEp*::kfp02-kfp04</i> ; <i>pepMppdAB</i> overexpression	This work
pDS0106	pIJ10257 derivative, <i>ermEp*::kfp02-kfp04</i> ; <i>pepMppdAB</i> overexpression	This work
pDS0107	pGus21 derivative, gene replacement of <i>kfp02-kfp04</i> (<i>pepMppdAB</i>) with <i>neo</i>	This work

Table S4. Strains used in this study

Name	Description	Source
<i>Escherichia coli</i> ET12567/pUZ8002	Donor strain for conjugation of plasmids to <i>Kitasatospora</i> sp.	MacNeil et al. 1992, Paget et al. 1999 ^{35,36}
<i>Escherichia coli</i> DH5a DSM 6897	General cloning strain	DSM ^{37,38}
<i>Escherichia coli</i> WM6242	Phosphonate antimicrobial indicator microorganism	University of Illinois ²⁹
<i>Kocuria. rhizophila</i> DSM 348	Antimicrobial indicator microorganism	DSM
<i>Streptomyces albus</i> J1074	Non-phosphonate producer used as heterologous host for <i>pepMppdAB</i> overexpression	Chater and Wilde 1976, Chater and Carter 1979 ^{39,40}
<i>Streptomyces lividans</i> T7	Non-phosphonate producer used as heterologous host for <i>pepMppdAB</i> overexpression	Lussier et al. 2010 ⁴¹
<i>Kitasatospora fiedleri</i> DSM 114396	University of Tübingen collection Tü4103; type strain	Zimmermann et al. ¹
<i>Kitasatospora fiedleri</i> YM0107	<i>K. fiedleri</i> DSM 114396 <i>phiBT1attB::pDS0101</i> (<i>luxR</i> overexpression)	This work
<i>Kitasatospora fiedleri</i> YM0108	<i>K. fiedleri</i> DSM 114396 <i>phiC31 attB::pDS0102</i> (<i>luxR</i> overexpression)	This work

<i>Kitasatospora fiedleri</i> YM0128	<i>K. fiedleri</i> DSM 114396 phiC31 attB::pDS0105 (<i>pepMppdAB</i> overexpression)	This work
<i>Kitasatospora fiedleri</i> YM0129	<i>K. fiedleri</i> DSM 114396 phiBT1attB::pDS0106 (<i>pepMppdAB</i> overexpression)	This work
<i>Kitasatospora fiedleri</i> YM0167	<i>K. fiedleri</i> YM0107 phiC31 attB::pDS0105 (<i>luxR pepMppdAB</i> overexpression)	This work
<i>Kitasatospora fiedleri</i> YM0168	<i>K. fiedleri</i> YM0108 phiBT1attB::pDS0106 (<i>luxR pepMppdAB</i> overexpression)	This work
<i>Kitasatospora fiedleri</i> YM0173	<i>K. fiedleri</i> DSM 114396 $\Delta kfp02-04::neo$ (<i>pepM-ppdAB</i> null mutant)	This work
<i>Kitasatospora fiedleri</i> YM0178	<i>K. fiedleri</i> YM0173 phiBT1attB::pDS0106 (complementation of <i>pepM-ppdAB</i> null mutant)	This work
<i>Streptomyces albus</i> YM0158	<i>Streptomyces albus</i> J1074 phiC31 attB::pDS0105 (<i>pepMppdAB</i> overexpression)	This work
<i>Streptomyces albus</i> YM0159	<i>Streptomyces albus</i> J1074 phiBT1attB::pDS0106 (<i>pepMppdAB</i> overexpression)	This work
<i>Streptomyces lividans</i> YM0152	<i>Streptomyces lividans</i> T7 phiC31 attB::pDS0105 (<i>pepMppdAB</i> overexpression)	This work
<i>Streptomyces lividans</i> YM0153	<i>Streptomyces lividans</i> T7 phiBT1attB::pDS0106 (<i>pepMppdAB</i> overexpression)	This work

Table S5. Oligonucleotides used in this study

Name	Sequence (5'→ 3')	Description
M13F	CGCCAGGGTTTCCCAGTCACGAC	Universal primer for sequencing of insert in many <i>lacZ</i> -containing vectors
M13R	TCACACAGGAAACAGCTATGAC	Universal primer for sequencing of insert in many <i>lacZ</i> -containing vectors
JP503	GCGATCACCGACAAGCTC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP508	GGAAGTTCCACGGGGTGAT	PCR test for <i>pepM-ppdAB</i> mutant screening
JP525	TTCATATGCGTGCATTGGGCGA	Cloning of <i>kfp24</i> (putative LuxR-like regulator)
JP526	AAGCTTGAACGCCGAACCGGATATGT	Cloning of <i>kfp24</i> (putative LuxR-like regulator)
JP549	<u>TTCATATG</u> CACACGTCCAGCGTG	Cloning of <i>ppdB</i> , <i>ppdA</i> , and <i>pepM</i> (<i>kfp02</i> , <i>kfp03</i> , and <i>kfp04</i>) with NdeI site
JP550	AAA <u>AAGCTT</u> GCTCCATCGTGATCCCTTC	Cloning of <i>ppdB</i> , <i>ppdA</i> , and <i>pepM</i> (<i>kfp02</i> , <i>kfp03</i> , and <i>kfp04</i>); HindIII underlined
JP551	TT <u>GGATCCT</u> GGCGCTGGTGCTGTTCAAG	Cloning of homologous region downstream of <i>kfp02-04</i> (<i>pepM-ppdAB</i>); BamHI underlined
JP552	AA <u>ACTAGT</u> CCACGAAGGGATCACGATGG	Cloning of homologous region downstream of <i>kfp02-04</i> (<i>pepM-ppdAB</i>); SpeI underlined
JP553	TTGACCTTCGTCGGTCGGTTC	Cloning of homologous region upstream of <i>kfp02-04</i> (<i>pepM-ppdAB</i>)
JP554	TT <u>CTCGAGAGG</u> TCGGCAAGACCACCTTC	Cloning of homologous region upstream of <i>kfp02-04</i> (<i>pepM-ppdAB</i>); XhoI underlined

JP555	GCTCCATCGTGATCCCTTC	Cloning of <i>ppdB</i> , <i>ppdA</i> , and <i>pepM</i> (<i>kfp02</i> , <i>kfp03</i> , and <i>kfp04</i>)/ PCR test for <i>pepM-ppdAB</i> mutant screening
JP556	GCTGGTGTCTGCAAGAC	Cloning of <i>ppdB</i> , <i>ppdA</i> , and <i>pepM</i> (<i>kfp02</i> , <i>kfp03</i> , and <i>kfp04</i>)/ PCR test for <i>pepM-ppdAB</i> mutant screening
JP569	CCCTGGTTGGCGTAGATCAC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP570	GAACGGTTCCGAGGTCATCC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP571	CTCGACGAAGCTGTTGAG	PCR test for <i>pepM-ppdAB</i> mutant screening
JP572	ATGTCCAAGAGCCGTACC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP573	ATGCCGTTGTCGACCACCAC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP574	GTGGTCCTGATGCAGAACTC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP575	TCTCGTGCATGCCGTTGTC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP576	CGTGGTCCTGATGCAGAACTC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP581	GGCTACCCGTGATATTGC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP582	GCGGTGATGATGATCGAG	PCR test for <i>pepM-ppdAB</i> mutant screening
JP583	CCTCGTCCTGCAGTTCATTC	PCR test for <i>pepM-ppdAB</i> mutant screening
JP584	ACTCCACCACGTCGATCTAC	PCR test for <i>pepM-ppdAB</i> mutant screening
pepMupfw	ATGAATTCTTCCCGGAGCTGGTGTCTG	PCR test for <i>pepM-ppdAB</i> mutant screening
pepMuprv	ATTCTAGAGGATGACCTCGGAACCGTTC	PCR test for <i>pepM-ppdAB</i> mutant screening
RTpepM2fw	CTGAACGGTTCCGAGGTC	PCR test for <i>pepM-ppdAB</i> mutant screening
RTpepM2rv	TGGTACGAGCGGACCATGTG	PCR test for <i>pepM-ppdAB</i> mutant screening

Table S6. Proposed phosphonate biosynthetic gene cluster from *Kitasatospora fiedleri* DSM 114396, based on the chromosome sequence with NCBI accession NZ_OX419519.1

Gene	Locus Tag	From position	To position	Strand	Size (bp)	Size (aa)	Genbank annotated product [new assigned gene and function]	Match
<i>kfp01</i>	QMQ26_RS15690	3401998	3403470	c	1473	490	aldehyde dehydrogenase [<i>adh</i> gene, phosphonoacetaldehyde dehydrogenase]	Adh
<i>kfp02</i>	QMQ26_RS15695	3403515	3404408	c	894	297	isocitrate lyase/phosphoenolpyruvate mutase family protein [<i>pepM</i> gene, PEP mutase]	PepM
<i>kfp03</i>	QMQ26_RS15700	3404459	3405058	c	600	199	thiamine pyrophosphate-dependent enzyme [<i>ppdA</i> gene, phosphonopyruvate decarboxylase subunit A]	PpdA
<i>kfp04</i>	QMQ26_RS15705	3405058	3405582	c	525	174	thiamine pyrophosphate-binding protein [<i>ppdB</i> gene, phosphonopyruvate decarboxylase subunit A]	PpdB
<i>kfp05</i>	QMQ26_RS15710	3405626	3406912	c	1287	428	acetyl-CoA carboxylase biotin carboxylase subunit family protein	
<i>kfp06</i>	QMQ26_RS15715	3406909	3407958	c	1050	349	hypothetical protein	
<i>kfp07</i>	QMQ26_RS15720	3408011	3408868	c	858	285	TauD/TfdA family dioxygenase	
<i>kfp08</i>	QMQ26_RS15725	3408894	3409289	c	396	131	hypothetical protein	
<i>kfp09</i>	QMQ26_RS15730	3409286	3410608	c	1323	440	cation:proton antiporter	
<i>kfp10</i>	QMQ26_RS15735	3410601	3411887	c	1287	428	sulfate adenylyltransferase subunit 1	CysN
<i>kfp11</i>	QMQ26_RS15740	3411890	3412810	c	921	306	sulfate adenylyltransferase subunit CysD	CysD
<i>kfp12</i>	QMQ26_RS15745	3412807	3413361	c	555	184	adenylyl-sulfate kinase CysC	CysC
<i>kfp13</i>	QMQ26_RS15750	3413358	3415343	c	1986	661	inositol monophosphatase family protein	CysQ
<i>kfp14</i>	QMQ26_RS15755	3415309	3416571	c	1263	420	MFS transporter	
<i>kfp15</i>	QMQ26_RS15760	3416682	3417728	c	1047	348	aspartate carbamoyltransferase PyrB	
<i>kfp16</i>	QMQ26_RS15765	3417845	3418054	c	210	69	hypothetical protein	
<i>kfp17</i>	QMQ26_RS15770	3418097	3418969	c	873	290	TauD/TfdA family dioxygenase	
<i>kfp18</i>	QMQ26_RS15775	3419045	3420304	c	1260	419	hypothetical protein	
<i>kfp19</i>	QMQ26_RS15780	3420279	3422249	c	1971	656	S9 family peptidase	
<i>kfp20</i>	QMQ26_RS15785	3422246	3423256	c	1011	336	histidinol-phosphate transaminase	
<i>kfp21</i>	QMQ26_RS15790	3423272	3424531	c	1269	422	hypothetical protein	
<i>kfp22</i>	QMQ26_RS15795	3424717	3425499	c	783	260	hypothetical protein	
<i>kfp23</i>	QMQ26_RS15800	3425565	3425738	c	174	57	hypothetical protein	
<i>kfp24</i>	QMQ26_RS15805	3426408	3427439	c	1032	343	LuxR C-terminal-related transcriptional regulator	
<i>kfp25</i>	QMQ26_RS15810	3427581	3428393	c	813	271	NUDIX domain-containing protein	

REFERENCES

1. Zimmermann A, Nouioui I, Pötter G, Neumann-Schaal M, Wolf J, Wibberg D, et al. *Kitasatospora fiedleri* sp. nov., a novel antibiotic-producing member of the genus *Kitasatospora*. *International Journal of Systematic and Evolutionary Microbiology*. 2023;73(11):006137.
2. Bibb MJ, Findlay PR, Johnson MW. The relationship between base composition and codon usage in bacterial genes and its use for the simple and reliable identification of protein-coding sequences. *Gene*. 1984 Oct 1;30(1):157–66.
3. Freestone TS, Ju KS, Wang B, Zhao H. Discovery of a Phosphonoacetic Acid Derived Natural Product by Pathway Refactoring. *ACS Synth Biol*. 2017 Feb 17;6(2):217–23.
4. Agarwal V, Peck SC, Chen JH, Borisova SA, Chekan JR, van der Donk WA, et al. Structure and function of phosphonoacetaldehyde dehydrogenase: the missing link in phosphonoacetate formation. *Chem Biol*. 2014 Jan 16;21(1):125–35.
5. Cioni JP, Doroghazi JR, Ju KS, Yu X, Evans BS, Lee J, et al. Cyanohydrin Phosphonate Natural Product from *Streptomyces regensis*. *J Nat Prod*. 2014 Feb 28;77(2):243–9.
6. Ju KS, Gao J, Doroghazi JR, Wang KKA, Thibodeaux CJ, Li S, et al. Discovery of phosphonic acid natural products by mining the genomes of 10,000 actinomycetes. *Proceedings of the National Academy of Sciences*. 2015 Sept 29;112(39):12175–80.
7. Takahashi E, Kimura T, Nakamura K, Arahira M, Iida M. Phosphonothrixin, a Novel Herbicidal Antibiotic Produced by *Saccharothrix* sp. ST-888 I. Taxonomy, Fermentation, Isolation and Biological Properties. *J Antibiot*. 1995 Oct 25;48(10):1124–9.
8. Bown L, Hirota R, Goettge MN, Cui J, Krist DT, Zhu L, et al. A Novel Pathway for Biosynthesis of the Herbicidal Phosphonate Natural Product Phosphonothrixin Is Widespread in Actinobacteria. *Journal of Bacteriology*. 2023 Apr 19;0(0):e00485-22.
9. Evans BS, Zhao C, Gao J, Evans CM, Ju KS, Doroghazi JR, et al. Discovery of the Antibiotic Phosacetamycin via a New Mass Spectrometry-Based Method for Phosphonic Acid Detection. *ACS Chem Biol*. 2013 May 17;8(5):908–13.
10. Nouioui I, Zimmermann A, Hennrich O, Xia S, Rössler O, Makitrynskyy R, et al. Challenging old microbiological treasures for natural compound biosynthesis capacity. *Front Bioeng Biotechnol*. 2024;12:1255151.
11. Kayrouz CM, Zhang Y, Pham TM, Ju KS. Genome Mining Reveals the Phosphonoalamide Natural Products and a New Route in Phosphonic Acid Biosynthesis. *ACS Chem Biol*. 2020 July 17;15(7):1921–9.
12. Circello BT, Eliot AC, Lee JH, Donk WA van der, Metcalf WW. Molecular Cloning and Heterologous Expression of the Dehydrophos Biosynthetic Gene Cluster. *Chemistry & Biology*. 2010 Apr 23;17(4):402–11.
13. Schwartz D, Recktenwald J, Pelzer S, Wohlleben W. Isolation and characterization of the PEP-phosphomutase and the phosphonopyruvate decarboxylase genes from the phosphinothricin

tripeptide producer *Streptomyces viridochromogenes* Tü494. FEMS Microbiology Letters. 1998 June 15;163(2):149–57.

14. Grammel N, Schwartz D, Wohlleben W, Keller U. Phosphinothricin-Tripeptide Synthetases from *Streptomyces viridochromogenes*. Biochemistry. 1998 Feb 1;37(6):1596–603.
15. Hidaka T, Shimotohno KW, Morishita T, Seto H. Studies on the biosynthesis of bialaphos (SF-1293). 18. 2-phosphinomethylmalic acid synthase: a descendant of (R)-citrate synthase? J Antibiot (Tokyo). 1999 Oct;52(10):925–31.
16. Polidore ALA, Caserio AD, Zhu L, Metcalf WW. Complete Biochemical Characterization of Pantaphos Biosynthesis Highlights an Unusual Role for a SAM-Dependent Methyltransferase. Angewandte Chemie International Edition. 2023;n/a(n/a):e202317262.
17. Woodyer RD, Shao Z, Thomas PM, Kelleher NL, Blodgett JAV, Metcalf WW, van der Donk WA, Zhao H. Heterologous Production of Fosfomycin and Identification of the Minimal Biosynthetic Gene Cluster. 2006. Chemistry & Biology 13, 1171–1182.
18. Hidaka T, Goda M, Kuzuyama T, Takei N, Hidaka M, Seto H. Cloning and nucleotide sequence of fosfomycin biosynthetic genes of *Streptomyces wedmorensis*. Molec Gen Genet. 1995 May 1;249(3):274–80.
19. Kobayashi S, Kuzuyama T, Seto H. Characterization of the *fomA* and *fomB* Gene Products from *Streptomyces wedmorensis*, Which Confer Fosfomycin Resistance on *Escherichia coli*. Antimicrob Agents Chemother. 2000 Mar;44(3):647–50.
20. Borisova SA, Circello BT, Zhang JK, van der Donk WA, Metcalf WW. Biosynthesis of Rhizocticins, Antifungal Phosphonate Oligopeptides Produced by *Bacillus subtilis* ATCC6633. Chemistry & Biology. 2010 Jan 29;17(1):28–37.
21. Cui J, Ju KS. Biosynthesis of *Bacillus* Phosphonoalamides Reveals Highly Specific Amino Acid Ligation. ACS Chem Biol. 2024 July 19;19(7):1506–14.
22. Yu X, Price NPJ, Evans BS, Metcalf WW. Purification and Characterization of Phosphonoglycans from *Glycomyces* sp. Strain NRRL B-16210 and *Stackebrandtia nassauensis* NRRL B-16338. J Bacteriol. 2014 May;196(9):1768–79.
23. Omura S, Murata M, Hanaki H, Hinotozawa K, Oiwa R, Tanaka H. Phosalacine, a new herbicidal antibiotic containing phosphinothricin. Fermentation, isolation, biological activity and mechanism of action. J Antibiot (Tokyo). 1984 Aug;37(8):829–35.
24. Blodgett JA, Zhang JK, Yu X, Metcalf WW. Conserved biosynthetic pathways for phosalacine, bialaphos and newly discovered phosphonic acid natural products. J Antibiot. 2016 Jan;69(1):15–25.
25. Shoji J, Kato T, Hino H, Hattori T, Hirooka K, Matsumoto K, et al. Production of fosfomycin (phosphonomycin) by *Pseudomonas syringae*. J Antibiot (Tokyo). 1986 July;39(7):1011–2.

26. Zhang Y, Chen L, Wilson JA, Cui J, Roodhouse H, Kayrouz C, et al. Valinophos Reveals a New Route in Microbial Phosphonate Biosynthesis That Is Broadly Conserved in Nature. *J Am Chem Soc.* 2022 June 8;144(22):9938–48.
27. Parkinson EI, Erb A, Eliot AC, Ju KS, Metcalf WW. Fosmidomycin biosynthesis diverges from related phosphonate natural products. *Nat Chem Biol.* 2019 Nov;15(11):1049–56.
28. Iguchi E, Okuhara M, Kohsaka M, Aoki H, Imanaka H. Studies on new phosphonic acid antibiotics II. Taxonomic studies on producing organisms of the phosphonic acid and related compounds. *J Antibiot.* 1980;33(1):18–23.
29. Eliot AC, Griffin BM, Thomas PM, Johannes TW, Kelleher NL, Zhao H, et al. Cloning, Expression, and Biochemical Characterization of *Streptomyces rubellomurinus* Genes Required for Biosynthesis of Antimalarial Compound FR900098. *Chemistry & Biology.* 2008 Aug 25;15(8):765–70.
30. Hong HJ, Hutchings MI, Hill LM, Buttner MJ. The role of the novel Fem protein VanK in vancomycin resistance in *Streptomyces coelicolor*. *J Biol Chem.* 2005 Apr 1;280(13):13055–61.
31. Menges R, Muth G, Wohlleben W, Stegmann E. The ABC transporter Tba of *Amycolatopsis balhimycina* is required for efficient export of the glycopeptide antibiotic balhimycin. *Appl Microbiol Biotechnol.* 2007 Nov 1;77(1):125–34.
32. Ladwig N, Franz-Wachtel M, Hezel F, Soufi B, Macek B, Wohlleben W, et al. Control of Morphological Differentiation of *Streptomyces coelicolor* A3(2) by Phosphorylation of MreC and PBP2. *PLOS ONE.* 2015 Apr 30;10(4):e0125425.
33. Rodríguez-García A, Santamarta I, Pérez-Redondo R, Martín JF, Liras P. Characterization of a two-gene operon *epeRA* involved in multidrug resistance in *Streptomyces clavuligerus*. *Res Microbiol.* 2006 Aug;157(6):559–68.
34. Alting-Mees MA, Short JM. pBluescript II: gene mapping vectors. *Nucleic Acids Res.* 1989 Nov 25;17(22):9494–9494.
35. MacNeil DJ, Gewain KM, Ruby CL, Dezeny G, Gibbons PH, MacNeil T. Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilizing a novel integration vector. *Gene.* 1992 Feb 1;111(1):61–8.
36. Paget MSB, Chamberlin L, Atrih A, Foster SJ, Buttner MJ. Evidence that the Extracytoplasmic Function Sigma Factor σ^E Is Required for Normal Cell Wall Structure in *Streptomyces coelicolor* A3(2). *J Bacteriol.* 1999 Jan 1;181(1):204–11.
37. Bethesda Research Laboratories. BRL pUC Host: *E. Coli* DH5alpha Competent Cells'. 1986;Focus 8(2).
38. Grant SG, Jessee J, Bloom FR, Hanahan D. Differential plasmid rescue from transgenic mouse DNAs into *Escherichia coli* methylation-restriction mutants. *Proceedings of the National Academy of Sciences.* 1990 June;87(12):4645–9.

39. Chater KF, Wilde LC. Restriction of a bacteriophage of *Streptomyces albus* G involving endonuclease SalI. *Journal of Bacteriology*. 1976 Nov;128(2):644–50.
40. Chater KF, Carter AT. A New, Wide Host-range, Temperate Bacteriophage (R4) of *Streptomyces* and its Interaction with some Restriction-Modification Systems. *Microbiology*. 1979;115(2):431–42.
41. Lussier FX, Denis F, Shareck F. Adaptation of the Highly Productive T7 Expression System to *Streptomyces lividans*. *Applied and Environmental Microbiology*. 2010 Feb;76(3):967–70.