TITLE PAGE FOR SUPPLEMENTARY DATA OF RESEARCH ARTICLE ENTITLED

Pseudomonas rhizozeae sp. nov., originated from rhizosphere soil of corn field

Sándor Kocsubé[1,3], Dóra Anna Papp[1], Attila Farkas[2], Thu Huynh[1], Henrietta Allaga[1], András Szekeres[1], Csaba Vágvölgyi[1], Judit Ámon[1,4], Zsuzsanna Hamari[1], Mónika Varga[1] *

[1]Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
[2]HCEMM-USZ Functional Cell Biology and Immunology Advanced Core Facility, University of Szeged, Szeged, Hungary.
[3]HUN-REN-SZTE Fungal Pathomechanisms Research Group, University of Szeged, Szeged, Hungary
[4]HCEMM-USZ Fungal Pathogens Research Group, University of Szeged, Szeged, Hungary

*Corresponding author:
Mónika Varga, vargam@bio.u-szeged.hu

Content:

Supplementary Table S1. Soil characteristics and chemicals applied at the isolation site of *Pseudomonas rhizozeae* SZMC28357^T

Supplementary Fig. S1. Maximum likelihood phylogram based on concatenated alignments of *16S rRNA*, *rpoB* and *rpoD* sequences.

Supplementary Table S2. dDDH values.

Supplementary Fig. S2. Biosynthetic Gene Clusters (BGCs) identified in the genom of *Pseudomonas rhizozeae* SZMC 28357^T sp. nov. by antiSMASH.

Supplementary Fig. S3. HPLC-HRMS analysis of the salt-stressed SZMC 28357^T culture.

Supplementary Fig. S4. Serial dilution spot assay of *Pseudomonas aeruginosa*, *Pseudomonas rhizozea* and *Bacillus megaterium* on King's agar medium under visible light (panel A) and UV illumination (panel B).

Supplementary Table S3. Polar lipid and isoprenoid quinone profile of SZMC 28357^T

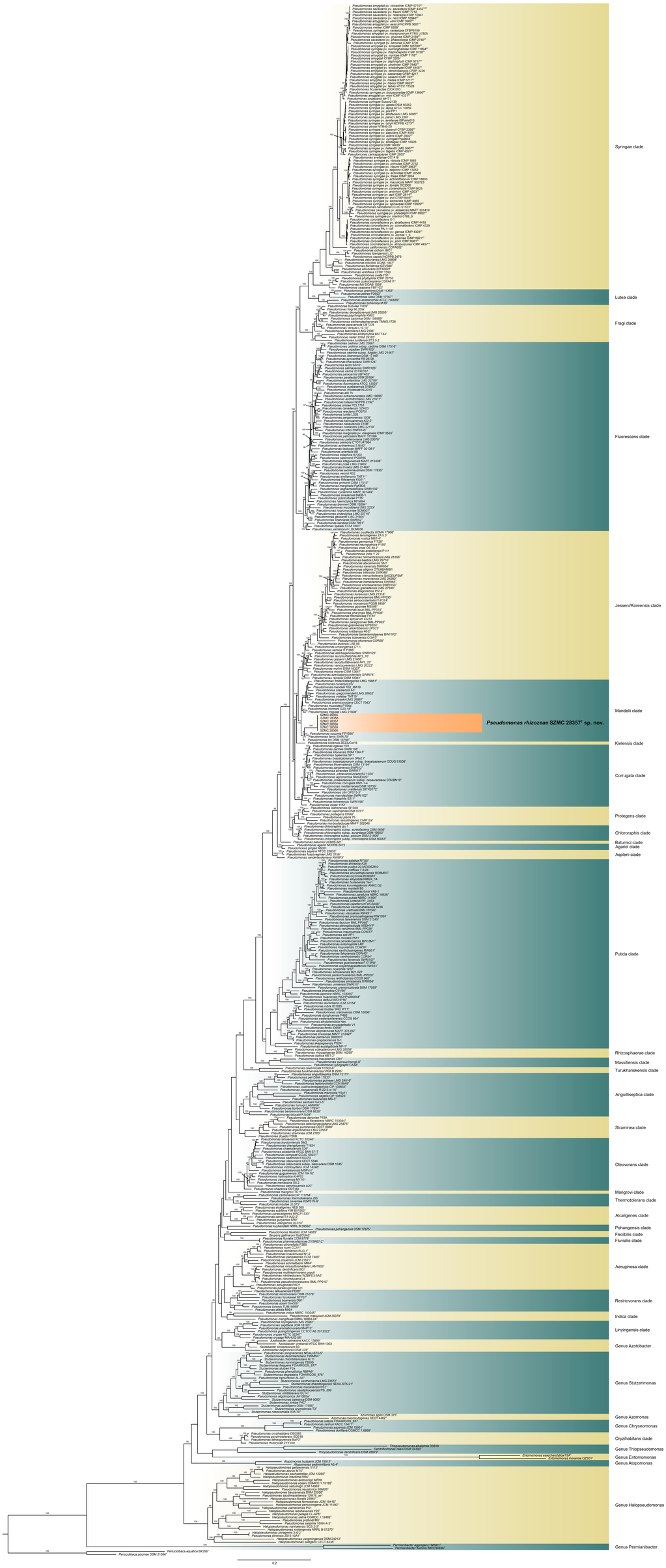
Supplementary Fig. S5. Heatmaps of ANI values between SZMC 28357^T and the most closely related genomes determined by phylogenomic analysis.

Supplementary methods. Analytical methods

References

Supplementary Table S1. Soil characteristics and chemicals applied at the isolation site of *Pseudomonas rhizozeae* SZMC28357^T

Geographic coordinates	46.08416° N, 19.26461° E
pH (KCl)	7.6
$CaCO_3$ content (w/w)	10.6%
humus (w/w)	1.84%
total soluble salts (w/w)	<0.02%
K_2O (mg/kg)	669
SO_4–S (mg/kg)	39
NOx–N (mg/kg)	11.1
Chemicals applied in the year of isolation	
Monocot-selective herbicide	40 g/L quizalofop-P-tefuril
Dicot-selective herbicide	40 g/l imazamox



Supplementary Fig. S1. Maximum likelihood phylogram based on concatenated alignments of 16S, *rpoB* and *rpoD* sequences. Clades were named after the publication of Rudra and Gupta et al. [1] and Girard et al. [2]. Statistical support of the best tree was calculated with UFBoot support. Only values higher than 95% are shown. Members of the genus *Perlucidibaca* were used as outgroup. Type strains and pathotypes are marked with superscript T and PT, respectively.

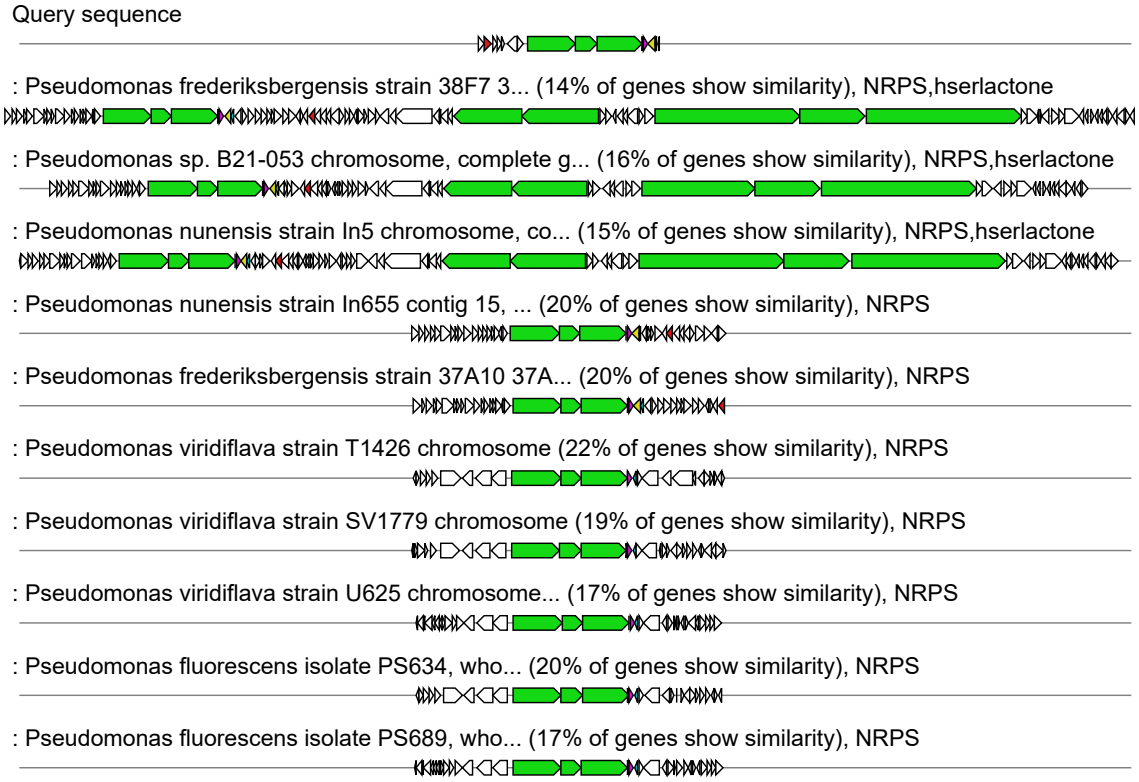
Supplementary Table S2. dDDH values.

Species	**G+C difference**	**dDDH (%)**
Pseudomonas agronomica SAICEU22^T	0.35	26.80
Pseudomonas alvandae SWRI17^T	0.08	26.20
Pseudomonas arsenicoxydans CECT 7543^T	2.02	27.70
Pseudomonas bijieensis SP1	1.47	26.50
Pseudomonas brassicacearum 3Re2-7	0.67	26.70
Pseudomonas brassicacearum subsp. *neoaurantiaca* CDVBN10	0.12	26.00
Pseudomonas canavaninivorans B21-020	0.06	26.20
Pseudomonas citri OPS13-3^T	0.02	26.20
Pseudomonas corrugata RM1-1-4	0.03	25.80
Pseudomonas cucumis FP1935^T	0.15	28.20
Pseudomonas farris SWRI79^T	0.11	27.80
Pseudomonas frederiksbergensis LMG 19851^T	1.78	28.30
Pseudomonas gregormendelii LMG 28632^T	2.03	27.30
Pseudomonas hormoni G20-18^T	1.85	28.30
Pseudomonas kielensis ZE23JCel16	1.18	27.30
Pseudomonas kilonensis DSM 13647^T	1.69	27.00
Pseudomonas lini DSM 16768^T	1.02	28.20
Pseudomonas mandelii KGI MA19	1.12	28.10
Pseudomonas marvdashtae SWRI102^T	0.38	26.20
Pseudomonas mediterranea DSM 16733^T	0.05	26.20
Pseudomonas migulae LMG 21608	0.63	28.30
Pseudomonas mucoides P154a^T	1.16	27.70
Pseudomonas nunensis In5^T	1.99	28.40
Pseudomonas ogarae FR1	1.48	26.70
Pseudomonas prosekii LMG 26867^T	0.13	28.00
Pseudomonas rhizophila S211^T	0.43	25.90
Pseudomonas silesiensis A3T	1.72	28.20
Pseudomonas tehranensis SWRI196^T	1.15	26.10
Pseudomonas thivervalensis DSM 13194^T	1.12	26.80
Pseudomonas uvaldensis 20TX0172^T	1.94	25.30
Pseudomonas viciae 11K1^T	1.39	26.40
Pseudomonas violetae TNT19^T	0.02	26.90
Pseudomonas zanjanensis SWRI12^T	1.21	26.70
Pseudomonas zarinae SWRI108^T	1.64	26.80

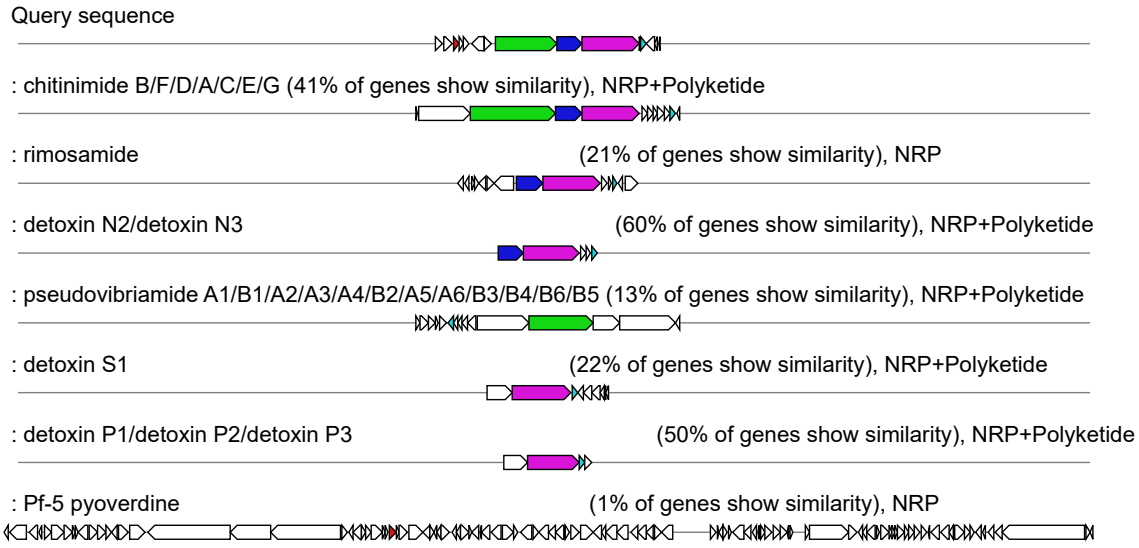
A

NZ_JAVLSB010000017.1 - Region 1 - NRPS (Region on contig edge.)
Location: 1 - 36,576 nt. (total: 36,576 nt)

ClusterBlast



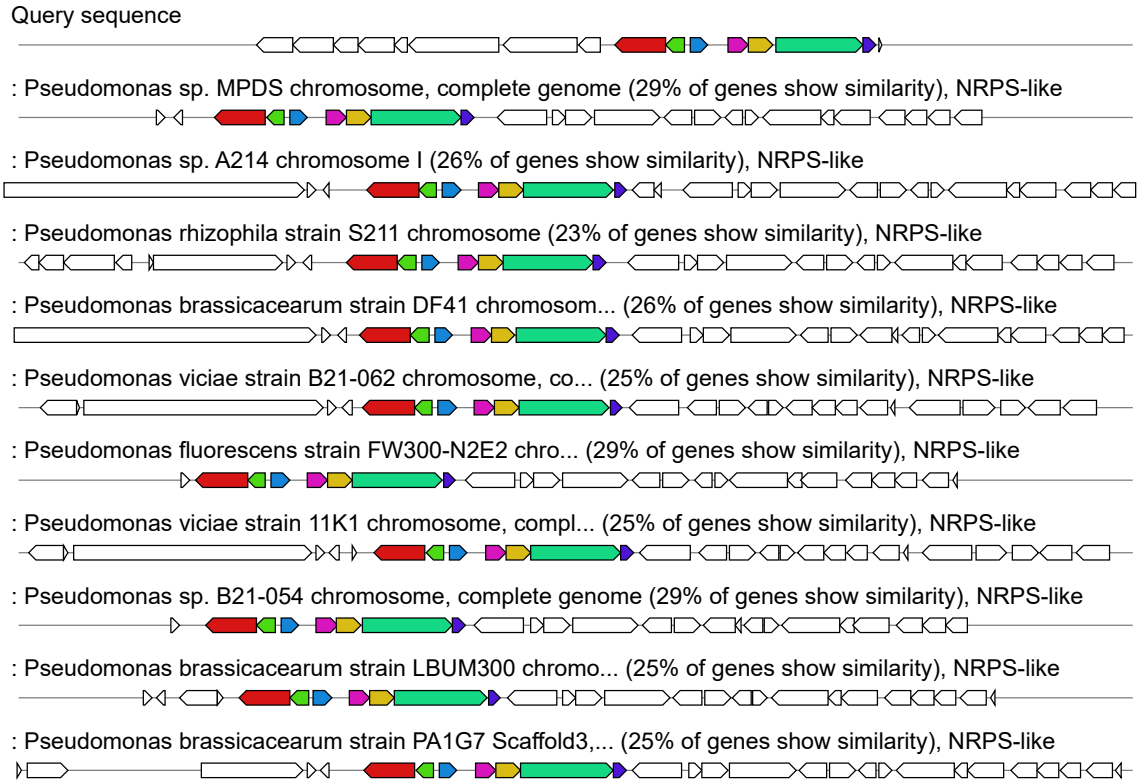
KnownClusterBlast



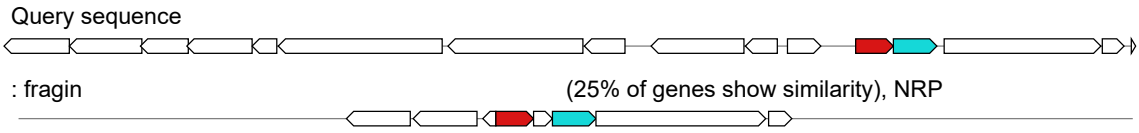
B

NZ_JAVLSB010000019.1 - Region 1 - NRPS-like (Region on contig edge.)
Location: 32,745 - 56,775 nt. (total: 24,031 nt)

ClusterBlast



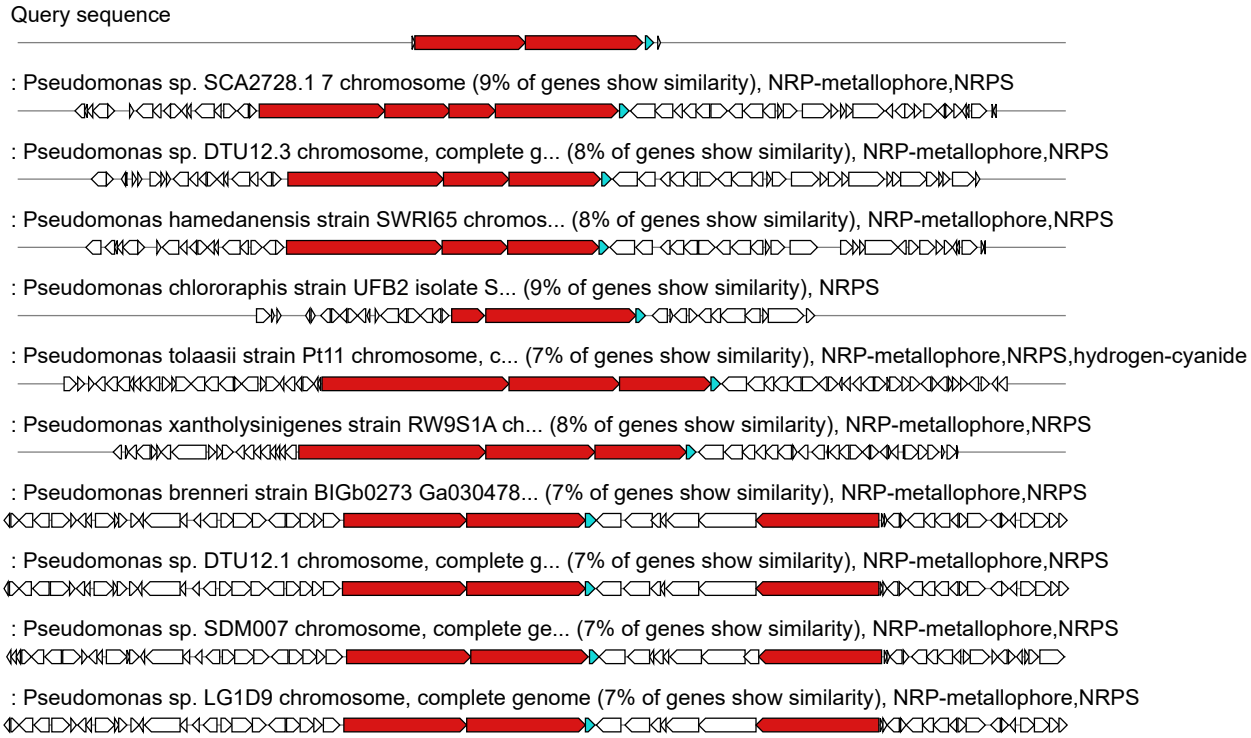
KnownClusterBlast



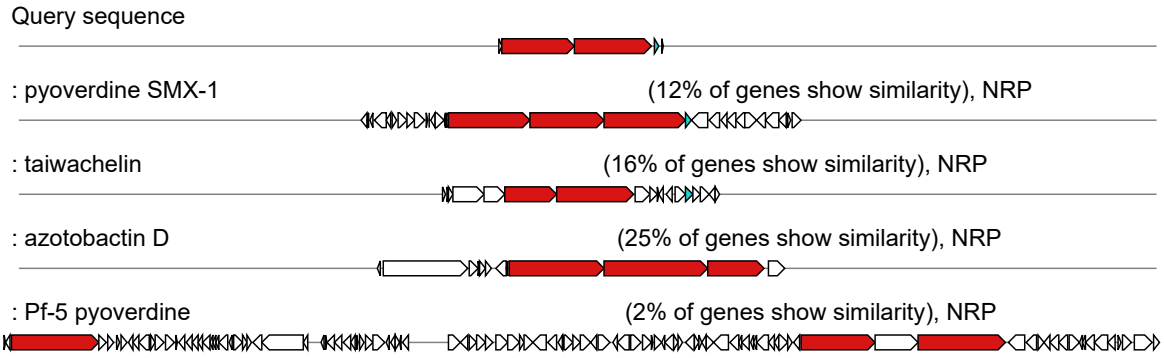
C

NZ_JAVLSB010000071.1 - Region 1 - NRPS (Region on contig edge.)
Location: 1 - 24,858 nt. (total: 24,858 nt)

ClusterBlast:



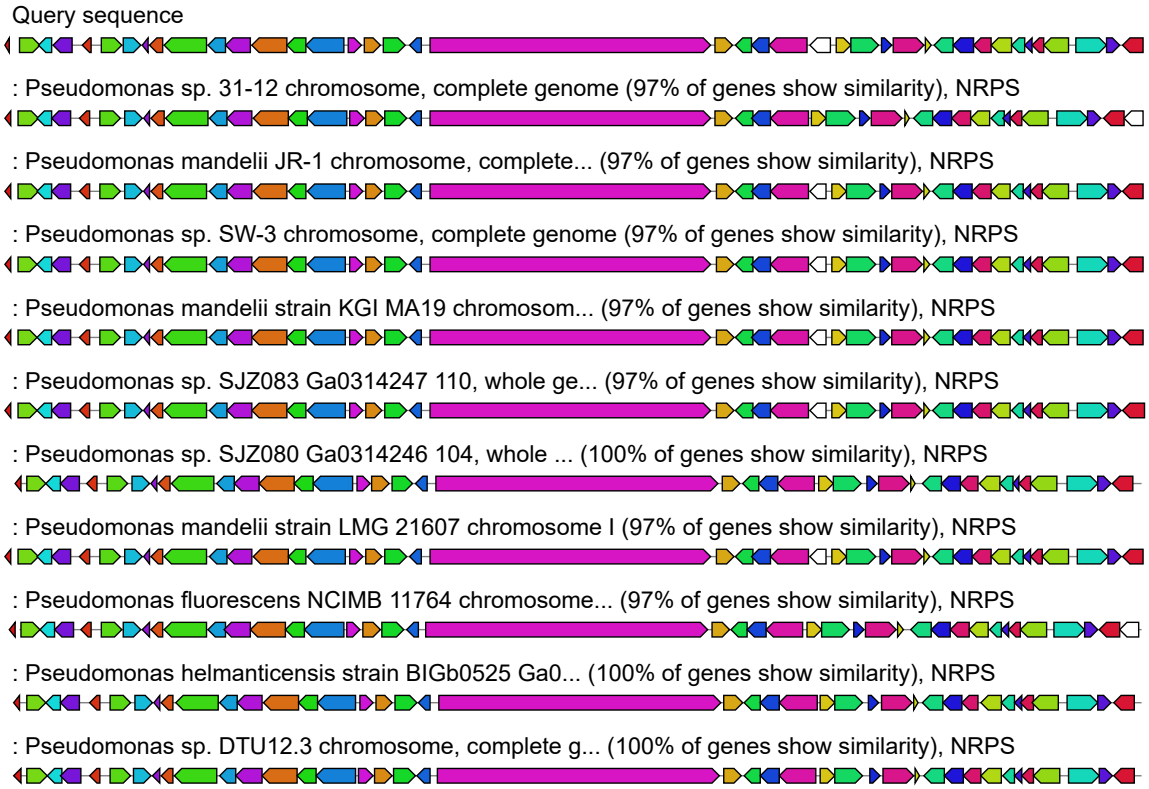
KnownClusterBlast:



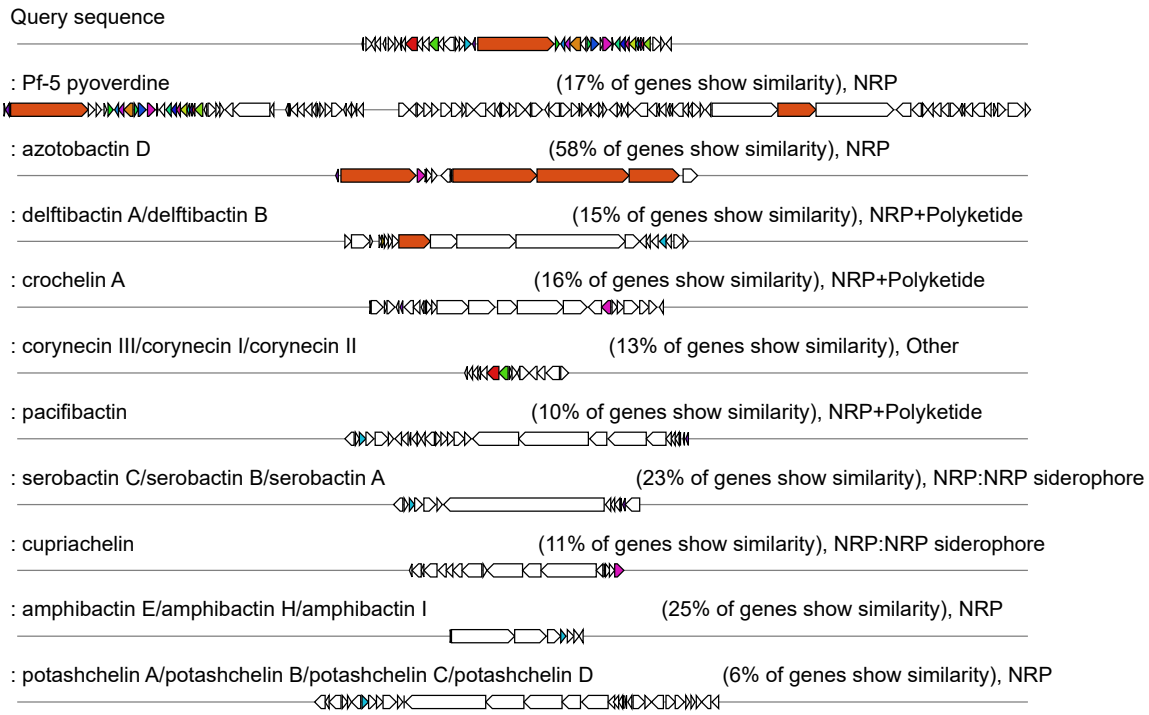
D

NZ_JAVLSB010000059.1 - Region 2 - NRPS
Location: 180,251 - 233,252 nt. (total: 53,002 nt)

ClusterBlast:



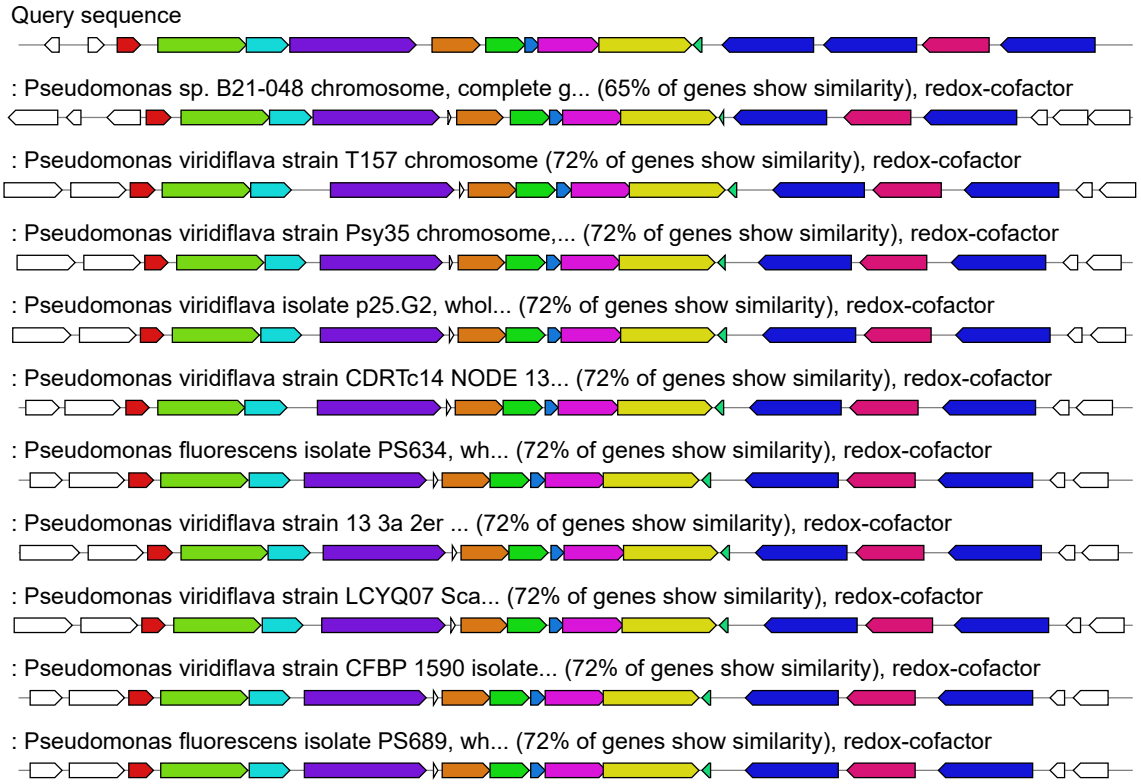
KnownClusterBlast:



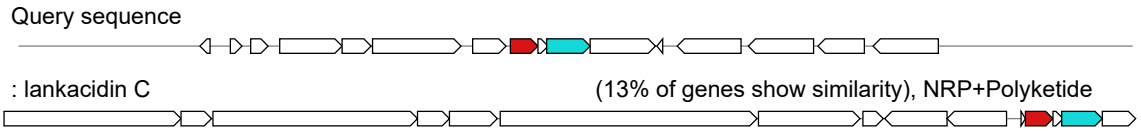
E

NZ_JAVLSB010000036.1 - Region 1 RiPP - redox-cofactor (Region on contig edge.)
Location: 76,020 - 98,185 nt. (total: 22,166 nt)

ClusterBlast:



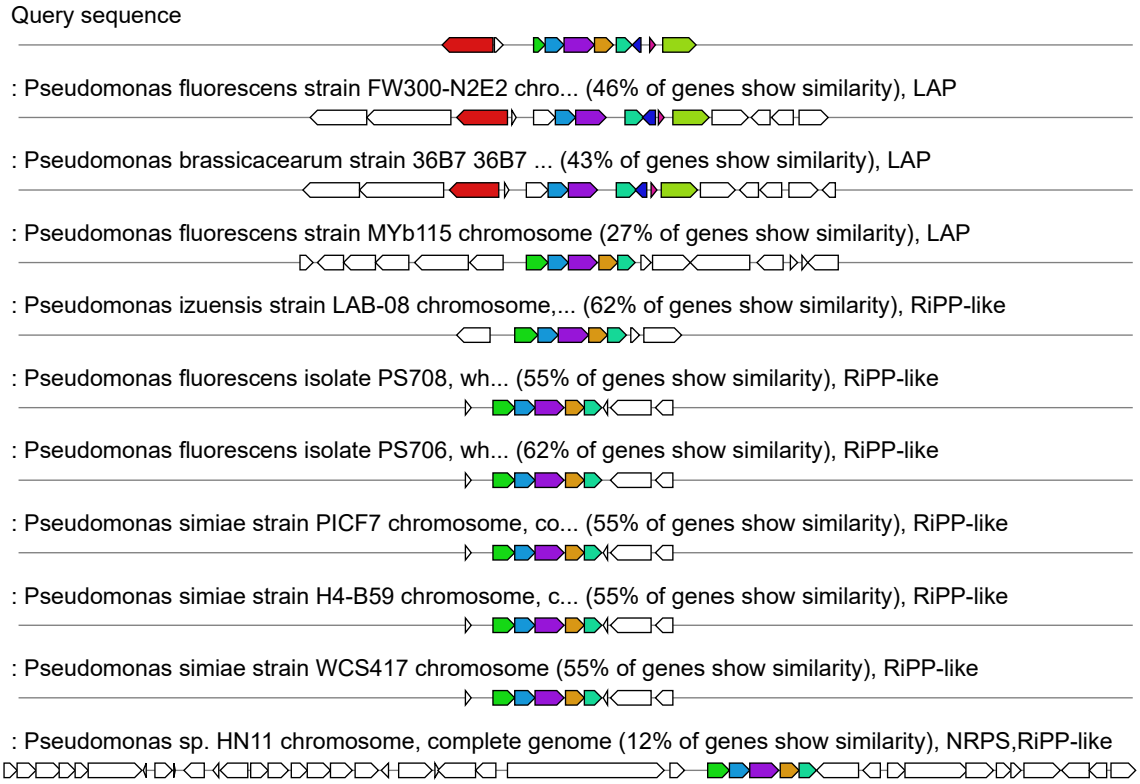
KnownClusterBlast:



F

NZ_JAVLSB010000031.1 - Region 1 - RiPP-like (Region on contig edge.)
Location: 24,558 - 35,745 nt. (total: 11,188 nt)

ClusterBlast:

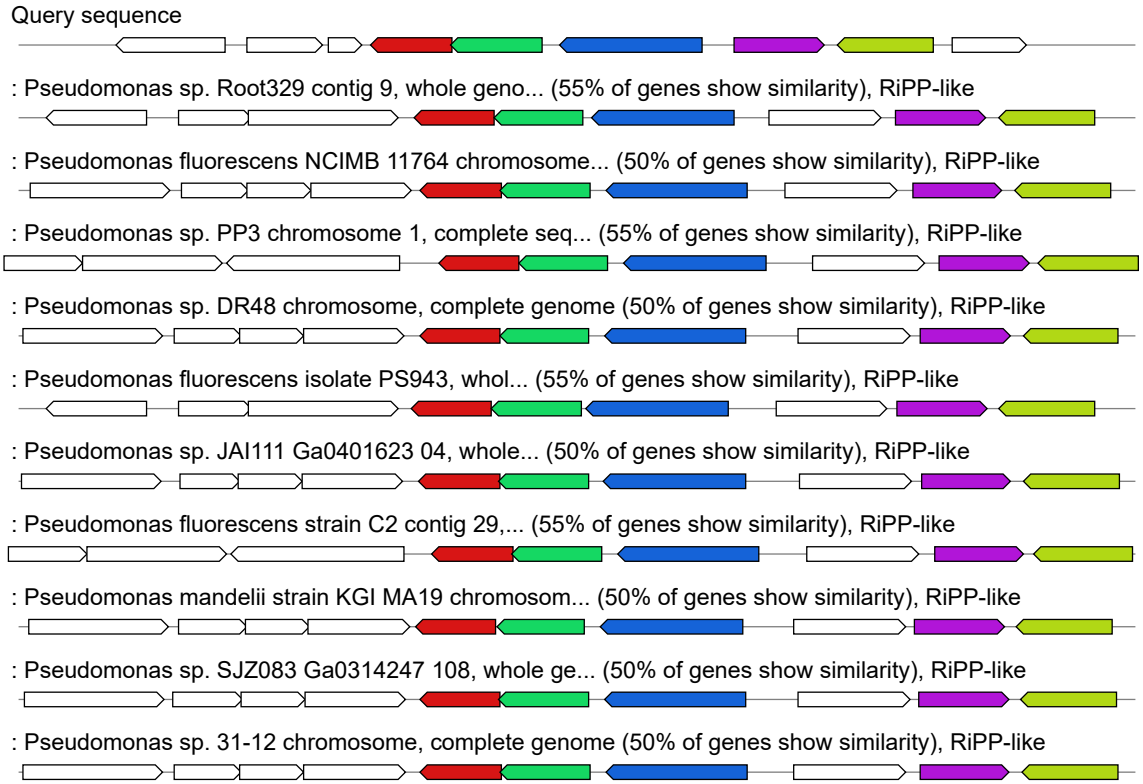


KnownClusterBlast: No matches found.

G

NZ_JAVLSB010000043.1 - Region 1 - RiPP-like
Location: 174,497 - 185,357 nt. (total: 10,861 nt)

ClusterBlast:

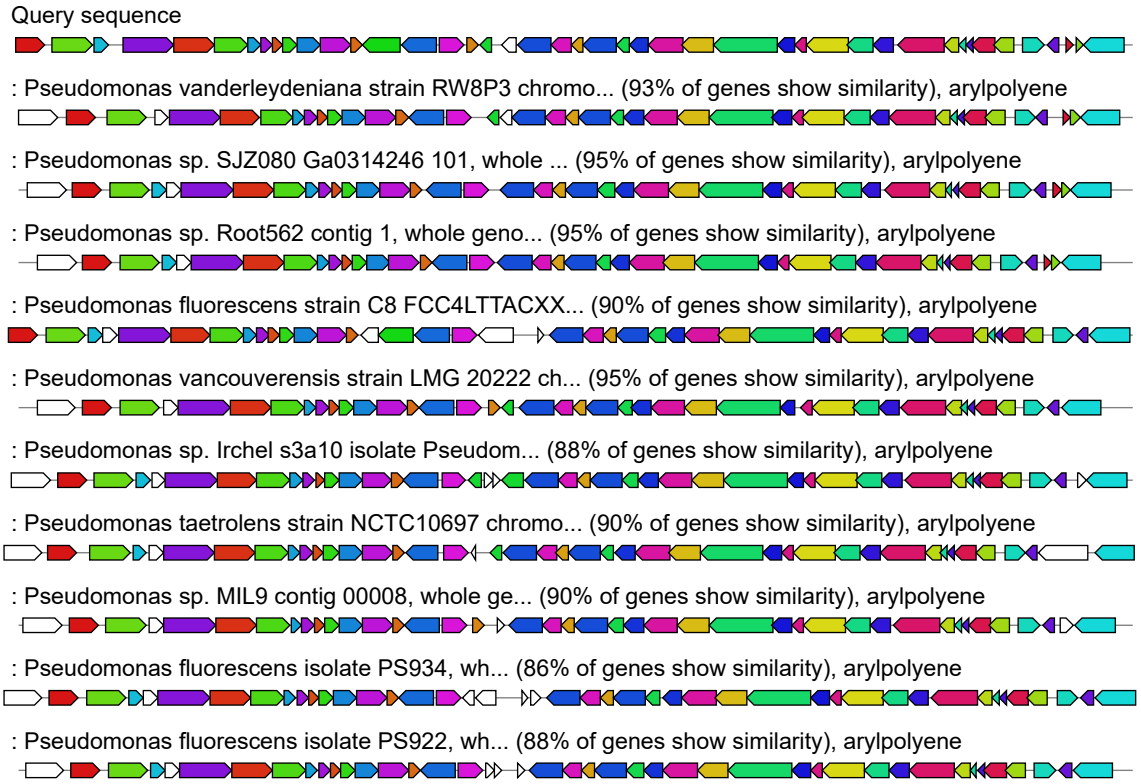


KnownClusterBlast: No matches found.

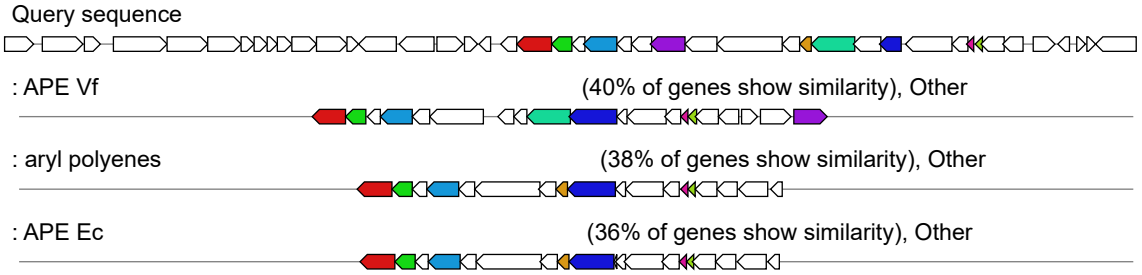
H

NZ_JAVLSB010000029.1 - Region 1 - PKS - arylpolyene
Location: 42,307 - 85,912 nt. (total: 43,606 nt)

ClusterBlast:



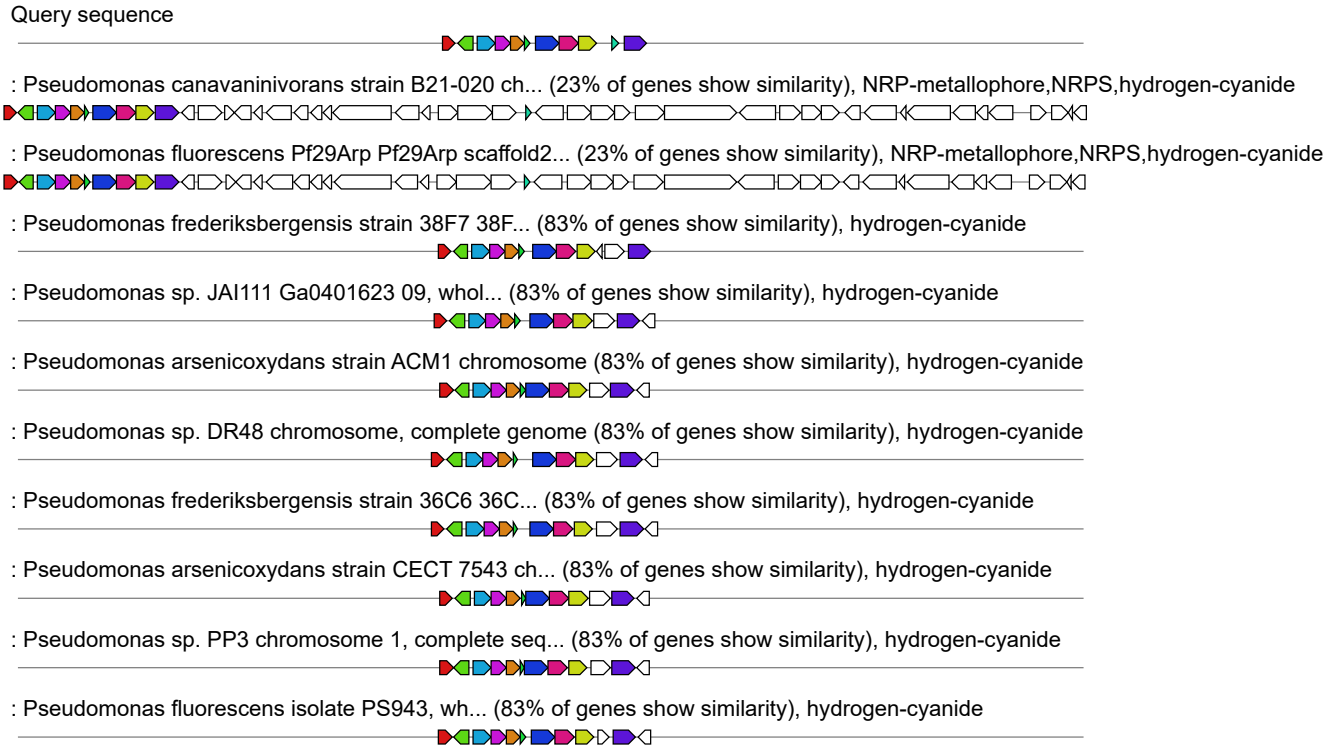
KnownClusterBlast:



I

NZ_JAVLSB010000022.1 - Region 1 Other - hydrogen-cyanide (Region on contig edge.)
Location: 34,175 - 46,392 nt. (total: 12,218 nt)

ClusterBlast:

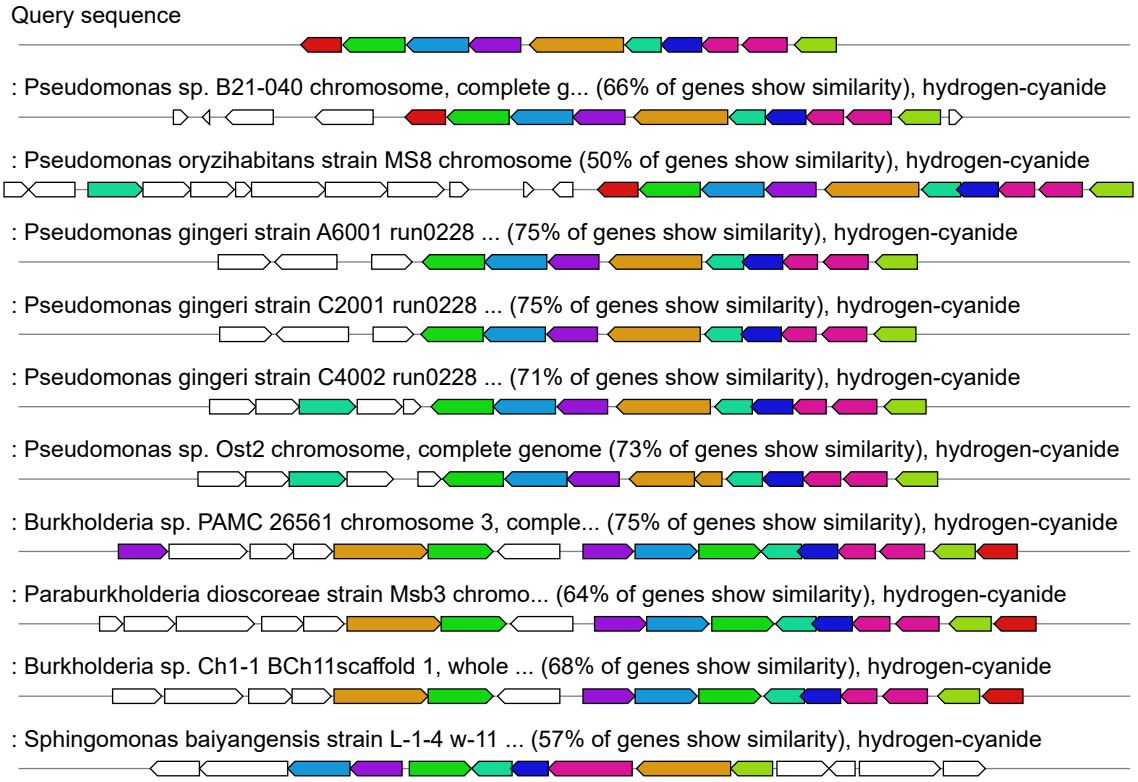


KnownClusterBlast: No matches found.

J

NZ_JAVLSB010000026.1 - Region 1 Other - hydrogen-cyanide (Region on contig edge.)
Location: 1 - 11,110 nt. (total: 11,110 nt)

ClusterBlast:

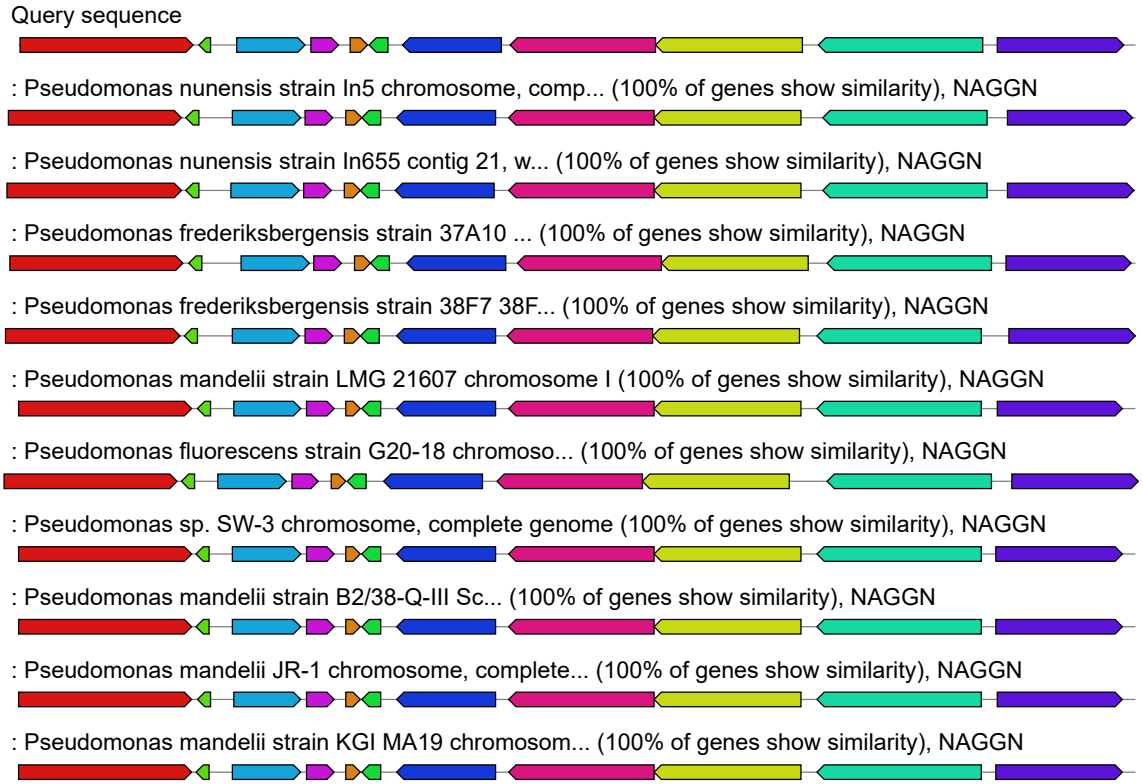


KnownClusterBlast: No matches found.

K

NZ_JAVLSB010000059.1 - Region 1 Other - NAGGN
Location: 36,334 - 51,157 nt. (total: 14,824 nt)

ClusterBlast:

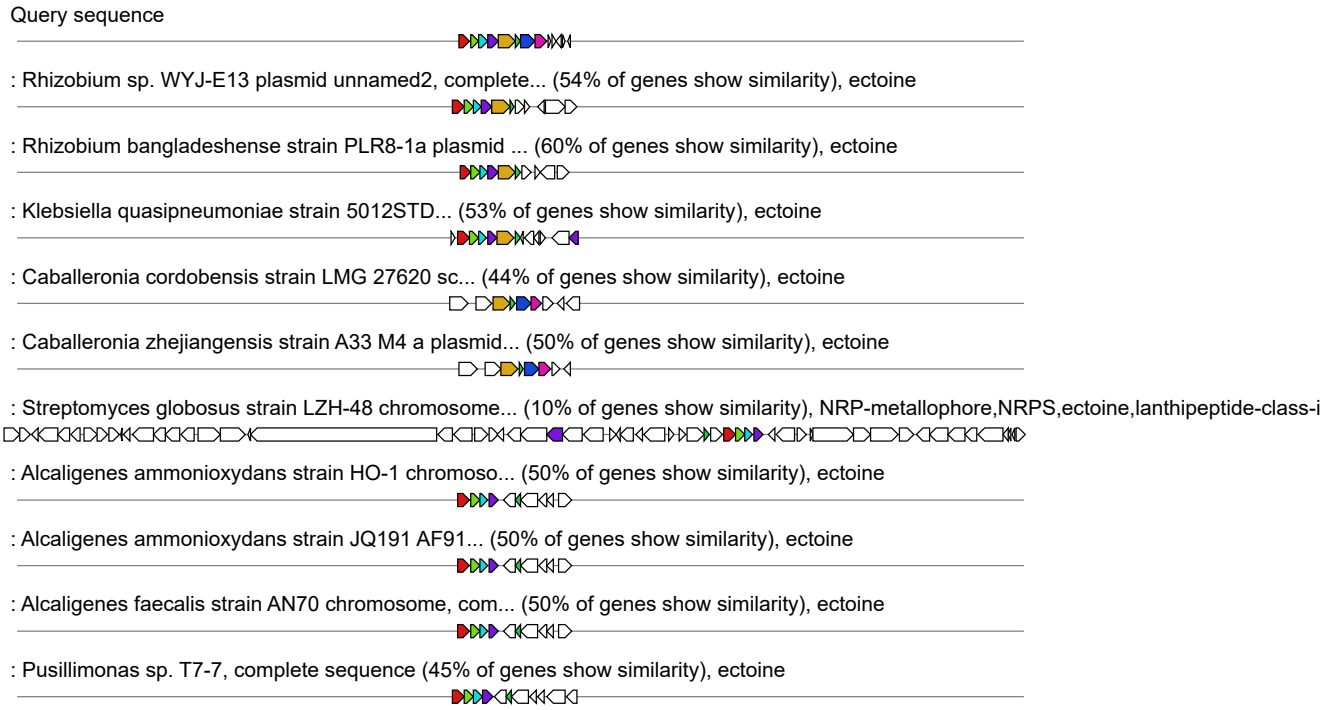


KnownClusterBlast: No matches found.

L

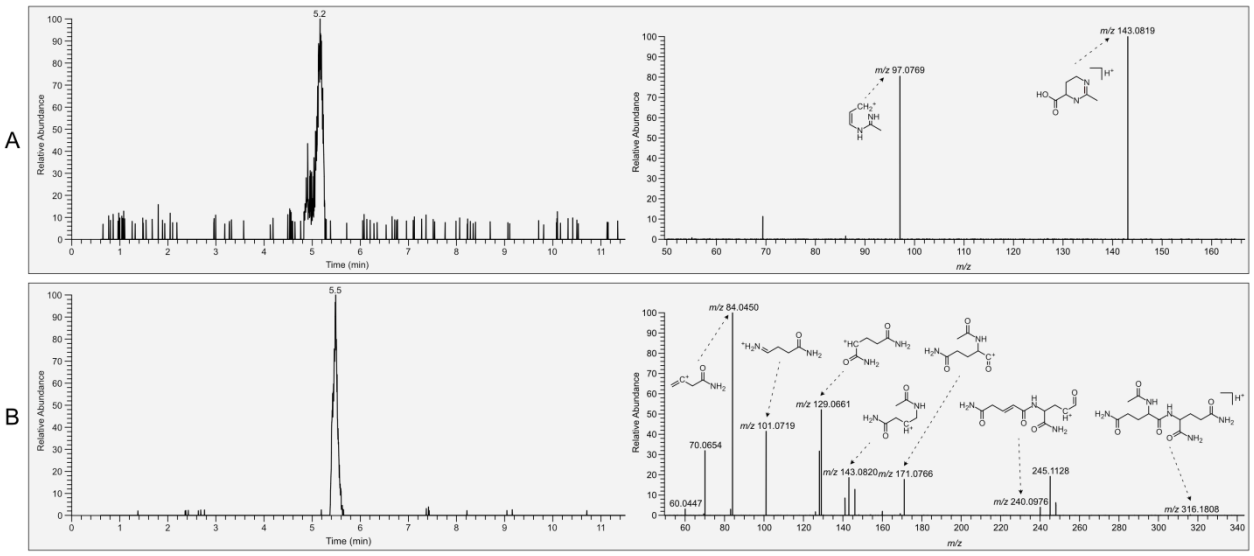
NZ_JAVLSB010000045.1 - Region 1 Other - ectoine
Location: 55,113 - 65,496 nt. (total: 10,384 nt)

ClusterBlast:

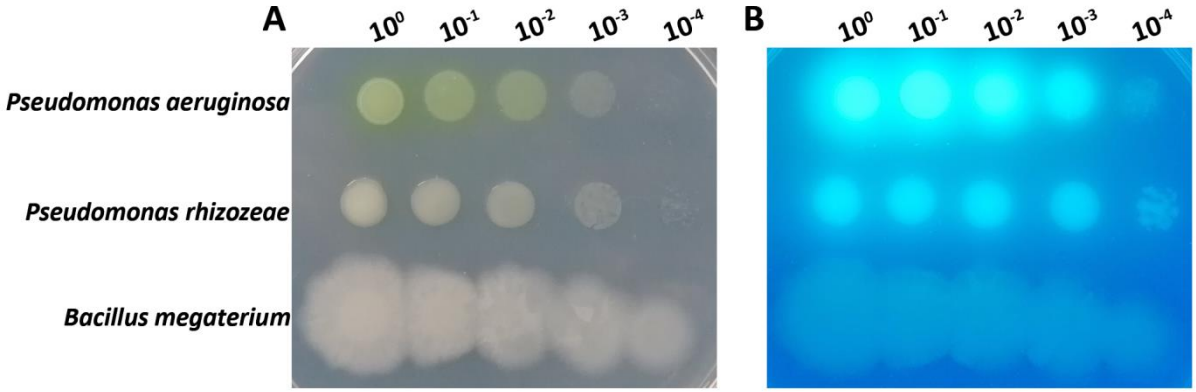


KnownClusterBlast: No matches found.

Supplementary Fig. S2. Biosynthetic Gene Clusters (BGCs) identified in the genom of *Pseudomonas rhizozeae* SZMC 28357^{T} sp. nov. by antiSMASH: **A**,**B**,**C** and **D** – NRPS clusters; **E**, **F** and **G** – RiPP clusters; **H** – PKS cluster; **I** and **J** – Hydrogen cyanide biosythetic clusters; **K** – NAGGN biosynthetic cluster; **L** – Ectoine biosynthetic cluster



Supplementary Fig. S3. HPLC-HRMS analysis of the salt-stressed SZMC 28357^{T} culture. (A): Extracted ion chromatogram and MS/MS spectra of ectoine $[M+H]^{+}$ *m/z* 143.0821. (B): Extracted ion chromatogram and MS/MS spectra with putative fragments of NAGGN $[M+H]^{+}$ *m/z* 316.1621.



Supplementary Fig. S4. Serial dilution spot assay of *Pseudomonas aeruginosa*, *Pseudomonas rhizozea* and *Bacillus megaterium* on King's agar medium under visible light (panel A) and UV illumination (panel B).

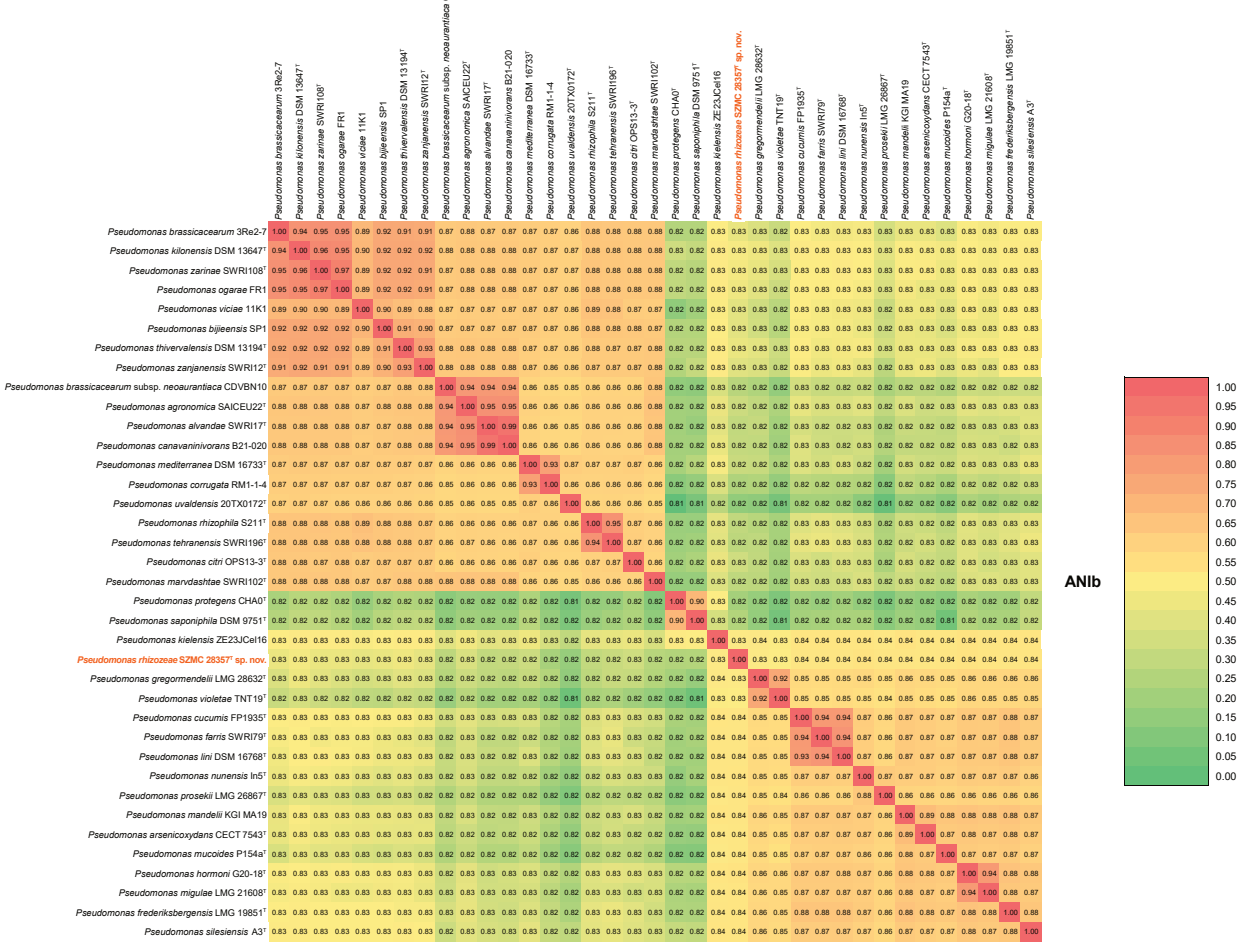
Tenfold serial dilutions of overnight cultures (LB broth, 30 °C, 16 h, 180 rpm) ranging from 10^0 to 10^{-4} (10^0 = undiluted culture) were spotted to assess growth and fluorescence intensity after 24 h incubation at 30 °C.

Supplementary Table S3. Polar lipid and isoprenoid quinone profile of SZMC 28357^{T}

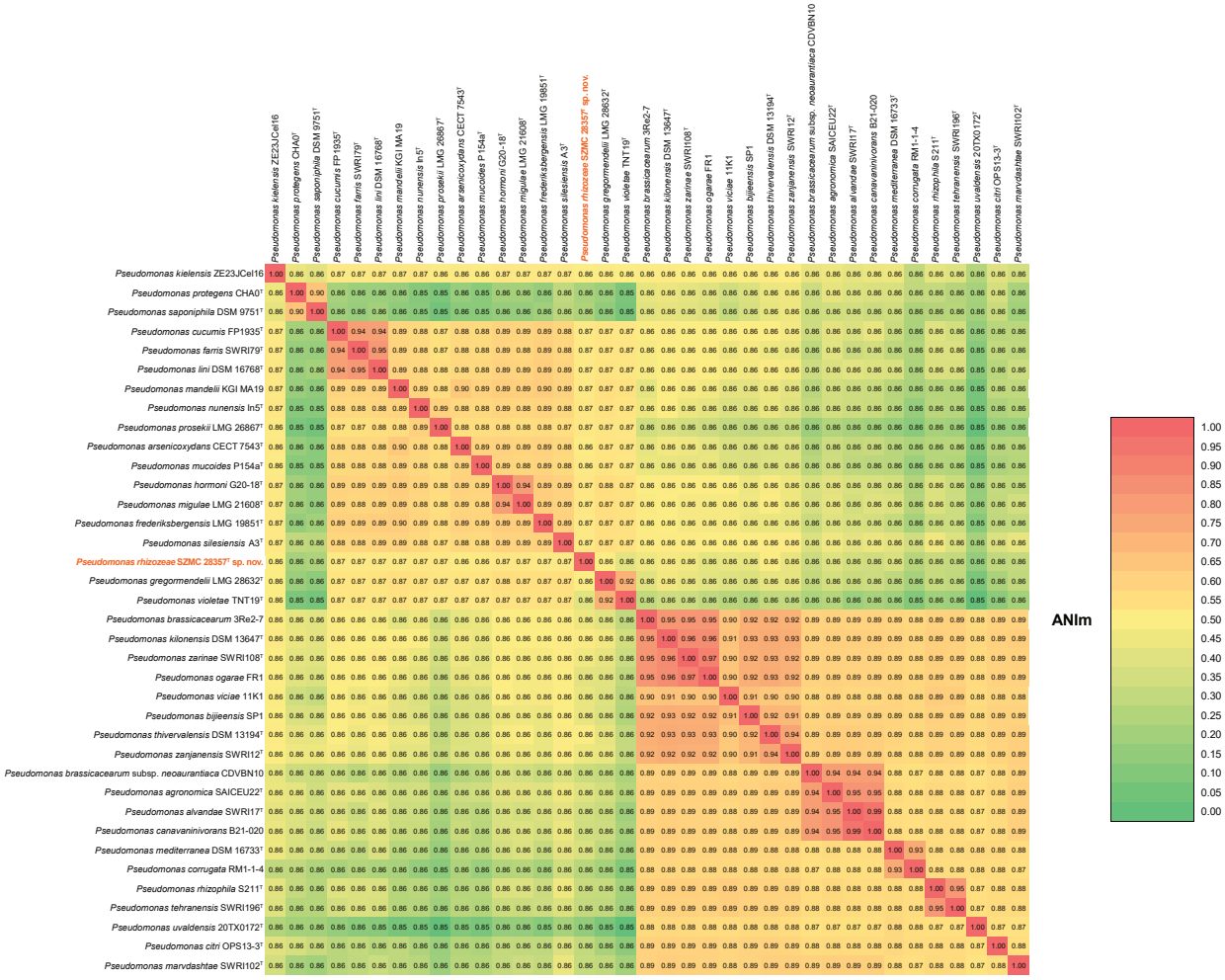
Compound species	Name	*m/z*	Reference ion	Retention time (min)	Abundance*
Phosphatidyl-choline	PC 18:1	522.3541	$[M+H]^{+1}$	6.8	6.97±6.34
	PC 37:7	790.5410	$[M+H]^{+1}$	16.6	8.86±7.97
	PC 37:0	804.6466	$[M+H]^{+1}$	17.5	8.85±8.07
	PC 38:0	818.6623	$[M+H]^{+1}$	17.9	8.12±7.22
Phosphatidyl-ethanolamine	PE 14:0_16:1	660.4618	$[M-H]^{-1}$	14.6	8.30±7.55
	PE 16:1_16:1	686.4773	$[M-H]^{-1}$	14.9	8.31±7.13
	PE 14:0_17:1	674.4773	$[M-H]^{-1}$	15.2	9.31±8.28
	PE 16:1_17:1	700.4929	$[M-H]^{-1}$	15.4	8.88±7.66
	PE 14:0_16:0	662.4776	$[M-H]^{-1}$	15.5	9.76±8.65
	PE 16:1_18:1	714.5085	$[M-H]^{-1}$	15.7	9.74±8.40
	PE 15:0_16:0	676.4930	$[M-H]^{-1}$	15.9	8.90±7.51
	PE 17:1_18:1	728.5243	$[M-H]^{-1}$	16.3	9.84±8.47
	PE 16:0_16:0	690.5087	$[M-H]^{-1}$	16.4	9.87±8.59
	PE 16:0_19:1	730.5401	$[M-H]^{-1}$	16.7	8.58±7.70
	PE 17:1_19:1	742.5401	$[M-H]^{-1}$	16.8	10.11±8.82
	PE 18:1_20:1	770.5716	$[M-H]^{-1}$	17.3	7.25±6.22
Phosphatidyl-glycerol	PG 16:1_17:1	731.4874	$[M-H]^{-1}$	13.7	8.76±7.77
	PG 14:0_16:0	693.4716	$[M-H]^{-1}$	13.7	9.77±8.88
	PG 16:0_16:1	719.4875	$[M-H]^{-1}$	13.9	10.01±8.94
	PG 15:0_16:0	707.4874	$[M-H]^{-1}$	14.3	8.90±8.02
	PG 17:1_17:1	745.5031	$[M-H]^{-1}$	14.3	9.49±8.53
	PG 16:0_17:1	733.5032	$[M-H]^{-1}$	14.4	10.5±9.44
	PG 17:1_18:1	759.5187	$[M-H]^{-1}$	14.6	9.90±8.84
	PG 16:0_18:1	747.5190	$[M-H]^{-1}$	14.8	10.07±8.75
	PG 17:1_19:1	773.5344	$[M-H]^{-1}$	15.2	9.81±7.55
	PG 16:0_18:0	749.5341	$[M-H]^{-1}$	15.6	9.40±8.43
Lysophosphatidyl ethanolamine	LPE 14:0	424.2478	$[M-H]^{-1}$	4.3	7.49±6.44
	LPE 17:1	464.2787	$[M-H]^{-1}$	5.9	8.42±7.59
	LPE 16:0	452.2788	$[M-H]^{-1}$	6.4	9.67±8.79
	LPE 18:1	478.2944	$[M-H]^{-1}$	6.8	9.04±8.10
	LPE 19:1	492.3104	$[M-H]^{-1}$	7.7	7.43±6.37
	LPE 19:1	492.3102	$[M-H]^{-1}$	8.3	8.67±7.69
	LPE 18:0	480.3102	$[M-H]^{-1}$	8.8	8.18±7.35
Lysophosphatidyl glycerol	LPG 14:0	455.2424	$[M-H]^{-1}$	3.2	7.02±6.99
	LPG 16:0	483.2734	$[M-H]^{-1}$	4.6	9.27±8.21
	LPG 18:1	509.2890	$[M-H]^{-1}$	4.9	8.72±7.53
	LPG 18:0	511.3044	$[M-H]^{-1}$	6.8	8.06±6.99
Isoprenoid quinone	Ubiquinone 8	744.5896	$[M+NH_4]^{+1}$	19.4	9.62±8.96
	Ubiquinone 4	455.3153	$[M+H]^{+1}$	20.8	8.55±6.59
	Ubiquinone 9	812.6540	$[M+NH_4]^{+1}$	20.8	10.54±9.60
	Ubiquinone 2	319.1916	$[M+H]^{+1}$	20.8	8.16±7.07

*Log-transformed abundance

A



B



Supplementary Fig. S5. Heatmaps of ANI values between SZMC 28357^{T} and the most closely related genomes determined by phylogenomic analysis. The values were obtained using pyANI-plus. ANI values lower than 95% indicate distinct species. Heatmap was generated by Excel using the raw tabulator separated output of pyANI-plus. **A** – ANIb values; **B** – ANIm values

Supplementary methods

Analytical methods

The isoprenoid quinones and polar lipids were determined using a DionexUltimate 3000 UHPLC system (Dionex) coupled to a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer equipped with a heated electrospray interface.

The quinones and polar lipids were separated using a Discovery HS C18 (3 µm, 150 x 2.1 mm) column. The mobile phases were 5 mM ammonium acetate (A) and methanol/2-propanol (1/1) containing 5 mM ammonium acetate (B) both supplemented with 0.1% acetic acid. The linear gradient was as follows: 0 min, 75% B; 2 min, 75% B; 14 min, 100% B; 19 min, 100% B; 19.5 min, 75% B; 20 min, 75% B. The flow rate was 0.2 mL/min, the injection volume was 5 µL and the column temperature was maintained at 45 °C. The quinones were analyzed in positive ionization mode and the polar lipids were analyzed both in positive and negative ionization mode.

The ion source had the following settings: the temperature of the probe heater and ion transfer capillary, spray voltage, sheath gas flow rate, auxiliary gas flow rate and S-lens RF level were set to 300 °C, 350 °C, 3.5 kV, 35 arbitrary unit, 10 arbitrary unit and 50 arbitrary unit, respectively. The mass spectrometer acquired data using a full-scan/data-dependent MS/MS method (Full MS/ddMS2). The full scan MS spectra were acquired at a resolution of 70,000 from *m/z* 100 to 1200 with a maximum injection time of 100 ms. For every full scan, 10 ddMS2-scans were carried out with a resolution of 17,500 and a minimum automatic gain control target of 1.00×10^5. Isolation window was 1.0 *m/z*. LC-HRMS data were acquired using Trace Finder 4.0 software. The raw MS data files were processed using Compound Discoverer™ (3.3) software.

References

[1] Rudra B, Gupta RS. Phylogenomics studies and molecular markers reliably demarcate genus *Pseudomonas sensu stricto* and twelve other *Pseudomonadaceae* species clades representing novel and emended genera. *Front Microbiol* 2024;14:1273665.

[2] Girard L, Lood C, Höfte M, Vandamme P, Rokni-Zadeh H, *et al.* The Ever-Expanding *Pseudomonas* Genus: Description of 43 New Species and Partition of the *Pseudomonas putida* Group. *Microorganisms* 2021;9:1766.