

Table S1. Designation of bacterial strains, year, host, and places where they were isolated.

Strains	Other designation	Year	Host	Obtained from	Country	State	City	NCBI Ac. N° 16S rRNA	NCBI Ac. N° <i>recA</i>
<i>N. bahiense</i>									
BR 11865 ^T	BRM 043117; YB 81	1999	<i>Brachiaria humidicola</i>	Rizosphere	Brazil	BA	Itabela	OR911837	PP025754
BR 11868	BRM 014147; YB 84	1999	<i>B. humidicola</i>	Rizosphere	Brazil	BA	Itabela	OR911836	PP025753
BR 11645	BRM 014078; Ym 69	1981	<i>Zea mays</i>	Roots	Brazil	RJ	Seropédica	OR911871	PP025655
BR 11644	BRM 014077; Ym 22	1981	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911872	PP025654
BR 11695	BRM 014079; 27	1998	<i>B. brizantha</i>	Rizosphere	Brazil	GO	Santo Antônio de Goiás	OR911870	PP025656
BR 11838	BRM 014117; YB 27	1998	<i>B. brizantha</i>	Rizosphere	Brazil	GO	Santo Antônio de Goiás	OR911846	PP025763
BR 11845	BRM 014124; YB 51	1999	<i>B. humidicola</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911845	PP025762
BR 11858	BRM 014137; YB 64	1999	<i>B. humidicola</i>	Rizosphere	Brazil	GO	Santo Antônio de Goiás	OR911838	PP025755
BR 11994	BRM 014195; YB 107	1999	<i>B. humidicola</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911813	PP025730
BR 11997	BRM 014198; YB 123	2000	<i>B. humidicola</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911812	PP025729
<i>N. guanabarensis</i>									
BR 11163 ^T	BRM 014038; YS 65	1981	<i>Sorghum vulgare</i>	Roots	Brazil	RJ	Seropédica	OR911896	PP025630
BR 11162	BRM 014037; YS 58	1981	<i>S. vulgare</i>	Rizosphere	Brazil	RJ	Seropédica	OR911897	PP025629
BR 11169	BRM 014044; YS 61	1981	<i>S. vulgare</i>	Roots	Brazil	RJ	Seropédica	OR911893	PP025633
<i>N. guaranorum</i>									
BR 11164 ^T	BRM 014039; YS 65	1981	<i>S. vulgare</i>	Rizosphere	Brazil	RJ	Seropédica	OR911895	PP025631
BR 11168	BRM 014043; YS 45	1981	<i>S. vulgare</i>	Rizosphere	Brazil	RJ	Seropédica	OR911894	PP025632
BR 11640	BRM 014073; Ym 36	1981	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911873	PP025653
BR 11170	BRM 014045; YS 69	1981	<i>S. vulgare</i>	Roots	Brazil	RJ	Seropédica	OR911892	PP025634
<i>N. guiasense</i>									
BR 11828 ^T	BRM 014107; Aam 24	2001	<i>Oryza sativa</i>	Roots	Brazil	RJ	Seropédica	OR911853	PP025751
BR 11870	BRM 014149; Aam 28	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911834	PP025672

BR 11755	BRM 014106; Aam 4	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911854	PP025772
BR 11754	BRM 014105; Aam 6	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911855	PP025657
BR 11736	BRM 014088; Aam 26	2000	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911869	PP025659
BR 11739	BRM 014090; Aam 13	2000	<i>O. sativa</i>	Aerial part	Brazil	GO	Santo Antônio de Goiás	OR911867	PP025665
BR 11746	BRM 014097; Aam 22	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911861	PP025666
BR 11747	BRM 014098; 2123A	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911860	PP025757
BR 11855	BRM 014134; Aam 43	2001	<i>O. sativa</i>	Aerial part	Brazil	RJ	Seropédica	OR911840	PP025739
BR 11938	BRM 014176; Aam 50	2000	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911822	PP025631

N. karajorum

BR 11752 ^T	BRM 014103; Aam 21	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911856	PP025670
BR 11737	BRM 014089; Aam 18	2000	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911868	PP025658
BR 11742	BRM 014093; Aam 1	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911864	PP025662
BR 11745	BRM 014096; Aam 8	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911862	PP025664
BR 11832	BRM 014111; Aam 59	2001	<i>O. sativa</i>	Aerial part	Brazil	RJ	Seropédica	OR911850	PP025767
BR 11872	BRM 014151; Aam 34	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911833	PP025750
BR 11850	BRM 014129; Aam 32	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911844	PP025761
BR 11853	BRM 014132; Aam 36	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911841	PP025758
BR 11869	BRM 014148; Aam 27	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911835	PP025752
BR 11873	BRM 014152; Am 42	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911832	PP025749
BR 11926	BRM 014164; Aam 10	2000	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911827	PP025744
BR 11928	BRM 014166; Aam 12	2000	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911826	PP025743
BR 11935	BRM 014173; Aam 41	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911824	PP025741

N. pindoramense

BR 11622 ^T	BRM 014055; Am 9	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911888	PP025638
BR 11991	BRM 014192; a152	1988	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911814	PP025731
BR 11967	BRM 014191; Am 34	2001	<i>Z. mays</i>	Xylem	Brazil	RJ	Seropédica	OR911815	PP025732
BR 11966	BRM 014190; Am 24	2001	<i>Z. mays</i>	Xylem	Brazil	RJ	Seropédica	OR911816	PP025733

BR 11835	BRM 014114; Aam 58	2001	<i>O. sativa</i>	Aerial part	Brazil	RJ	Seropédica	OR911847	PP025764
BR 11740	BRM 014091; 2134	2000	<i>O. sativa</i>	Aerial part	Brazil	RJ	Seropédica	OR911866	PP025660
BR 11639	BRM 014072; Am 20	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911874	PP025652
BR 11623	BRM 014056; Am 27	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911887	PP025639
BR 11637	BRM 014070; Am 34	2001	<i>Z. mays</i>	Xylem	Brazil	RJ	Seropédica	OR911875	PP025651
BR 11632	BRM 014062; Am 29	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911880	PP025646
BR 11628	BRM 014061; Am 28	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911883	PP025643
BR 11621	BRM 014054; Am 3	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911889	PP025637
BR 11629	BRM 014062; Am 5	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911882	PP025644
BR 11633	BRM 014066; Am 16	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911879	PP025647
BR 11624	BRM 014057; Am 7	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911886	PP025640
BR 11634	BRM 014067; Am 32	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911878	PP025648
BR 11748	BRM 014099; Aam 5	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911859	PP025667
BR 11627	BRM 014060; Am 19	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911884	PP025642
BR 11630	BRM 014063; Am 8	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911881	PP025645
BR 11636	BRM 014069; Am 30	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911876	PP025650
BR 11620	BRM 14053; Am 31	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911890	PP025636
BR 11625	BRM 014058; Am 25	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911885	PP025641
BR 11635	BRM 014068; Am 24	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911877	PP025649
BR 11834	BRM 014113; Aam 57	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911848	PP025765
BR 11877	BRM 014156; Aam 53	2001	<i>O. sativa</i>	Aerial part	Brazil	RJ	Seropédica	OR911828	PP025745
BR 11619	BRM 014052; Am 12	2001	<i>Z. mays</i>	Roots	Brazil	RJ	Seropédica	OR911891	PP025635
BR 11741	BRM 014092; Aam 2	2000	<i>O. sativa</i>	Aerial part	Brazil	GO	Santo Antônio de Goiás	OR911865	PP025661
BR 11750	BRM 014101; Aam 16	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911858	PP025668
BR 11751	BRM 014102; Aam 9	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911857	PP025669
BR 11829	BRM 014108; Aam 31	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911852	PP025769

BR 11830	BRM 014109; Aam 55	2001	<i>O. sativa</i>	Aerial part	Brazil	GO	Santo Antônio de Goiás	OR911851	PP025768
BR 11833	BRM 014112; Aam 6	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911849	PP025766
BR 11944	BRM 014182; Aam 67	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911820	PP025737
BR 11945	BRM 014183; Aam 69	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911819	PP025736
BR 11943	BRM 014181; Aam 66	2000	<i>B. brizantha</i>	Rizosphere	Brazil	GO	Santo Antônio de Goiás	OR911821	PP025738
BR 11743	BRM 014094; Aam 14	2001	<i>O. sativa</i>	Roots	Brazil	GO	Santo Antônio de Goiás	OR911863	PP025663
BR 11851	BRM 014130; Aam 33	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911843	PP025760
BR 11857	BRM 014136; Aam 49	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911839	PP025756
BR 11852	BRM 014131; Aam 35	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911842	PP025759
BR 11948	BRM 014186; Aam 76	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911818	PP025735
BR 11875	BRM 014154; Aam 51	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911830	PP025747
BR 11876	BRM 014155; Aam 52	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911829	PP025746
BR 11933	BRM 014171; Aam 39	2000	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911825	PP025742
BR 11949	BRM 014187; Aam 82	2001	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911817	PP025734
BR 11937	BRM 014175; Aam 47	2000	<i>O. sativa</i>	Roots	Brazil	RJ	Seropédica	OR911823	PP025740
BR 11874	BRM-014153; Aam 44	2001	<i>O. sativa</i>	Aerial part	Brazil	GO	Santo Antônio de Goiás	OR911831	PP025748
<i>N. amazonense</i>									
BR 11142 ^T	Y-1, AM 14, DSM 2787, ATCC 35119, BRM 006217	1981	<i>Digitaria decumbens</i>	Roots	Brazil	RJ	Seropédica	OQ557120)	OQ576776
<i>N. viridazoti</i>									
BR 11140 ^T	Y-2, Am 18, DSM 2788, ATCC 35120 BRM 006215	1981	<i>Hyparrhenia rufa</i>	Roots	Brazil	RJ	Seropédica	OQ557121	OQ576777
<i>N. iridis</i>									
DSM 22198 ^T	YC6995 ^T , BR 15444	-	<i>Iris ensata</i>	Rizosphere	Korea	-	-	GU048666)	GCA_014205765.1

Table S2. Results from Tajima's Neutrality Test

	m	S	p_s	θ	π	D
<i>16s</i>	90	857	0.555772	0.104859	0.182249	2.484956
<i>recA</i>	90	320	0.616570	0.118410	0.175001	1.609921

Table S3. Gene set for chemotaxonomy analyzes

Biochemical Pathway	Description	Pathway Map	Genome	BRC/PATRIC ID	AA Length
Glycerolipid and Glycerophospholipid Metabolism	Phosphate acyl-ACP acyltransferase PlsX	KEGG: 00564	<i>A. brasilense</i> BR 11669	fig 192.245.peg.75	350
			BR 11163 ^T	fig 1543705.6.peg.4781	356
			BR 11164 ^T	fig 1543705.9.peg.3614	358
			BR 11752 ^T	fig 1543705.7.peg.2860	356
			BR 11828 ^T	fig 1543705.12.peg.2570	356
			BR 11140 ^T	fig 1543705.23.peg.909	357
			BR 11142 ^T	fig 1543705.19.peg.2777	357
			BR 11622 ^T	fig 1543705.24.peg.1420	356
			BR 11865 ^T	fig 1543705.25.peg.1027	357
			DMS 22198 ^T	fig 1543705.22.peg.824	356
Fatty acid metabolism; Fatty acid elongation in mitochondria	3-ketoacyl-CoA thiolase 2 [fadN-fadA-fadE operon]	KEGG:00071 K EGG:00062	<i>A. brasilense</i> BR 11669	fig 192.245.peg.3367	396
			BR 11163 ^T	fig 1543705.6.peg.3246	397
			BR 11164 ^T	fig 1543705.9.peg.551	397
			BR 11752 ^T	fig 1543705.7.peg.3246	395
			BR 11828 ^T	fig 1543705.12.peg.52	395
			BR 11140 ^T	fig 1543705.23.peg.1517	397
			BR 11142 ^T	fig 1543705.19.peg.2358	395
			BR 11622 ^T	fig 1543705.24.peg.5293	395
			BR 11865 ^T	fig 1543705.25.peg.5724	395

			DMS 22198 ^T	fig 1543705.22.peg.4484	395
Fatty acid biosynthesis	Acyl-CoA thioesterase YciA	KEGG:00061	<i>A. brasilense</i> BR 11669	fig 192.245.peg.2316	480
			BR 11163 ^T	fig 1543705.6.peg.5014	396
			BR 11164 ^T	fig 1543705.9.peg.3383	396
			BR 11752 ^T	fig 1543705.7.peg.5101	396
			BR 11828 ^T	fig 1543705.12.peg.2374	396
			BR 11140 ^T	fig 1543705.23.peg.1071	396
			BR 11142 ^T	fig 1543705.19.peg.2995	396
			BR 11622 ^T	fig 1543705.24.peg.1224	396
			BR 11865 ^T	fig 1543705.25.peg.1554	396
			DMS 22198 ^T	fig 1543705.22.peg.104	396
Terpenoid backbone biosynthesis	Decaprenyl diphosphate synthase	KEGG:00900	<i>A. brasilense</i> BR 11669	fig 192.245.peg.3765	1017
			BR 11163 ^T	fig 1543705.6.peg.2812	1023
			BR 11164 ^T	fig 1543705.9.peg.5839	1023
			BR 11752 ^T	fig 1543705.7.peg.1048	1023
			BR 11828 ^T	fig 1543705.12.peg.4014	1023
			BR 11140 ^T	fig 1543705.23.peg.153	1023
			BR 11142 ^T	fig 1543705.19.peg.5738	1023
			BR 11622 ^T	fig 1543705.24.peg.54	1023
			BR 11865 ^T	fig 1543705.25.peg.3160	1023
			DMS 22198 ^T	fig 1543705.22.peg.5511	1023

Table S4. Ethylene production by *Nitrospirillum* strains proposed as new species and the type strains *N. iridis* DSM 22198^T, *N. viridazoti* BR 11140^T, and *N. amazonense* BR 11142^T grown in nitrogen-free LGI semi-solid medium, followed by incubation with acetylene.

Strains	Ethylene production (nmol of C ₂ H ₄ mL ⁻¹ h ⁻¹)
DSM 22198 ^T	496.2
BR 11140 ^T	459.6
BR 11142 ^T	521.6
BR 11163 ^T	648.6
BR 11164 ^T	574.2
BR 11622 ^T	520.1
BR 11752 ^T	504.8
BR 11828 ^T	644.7
BR 11865 ^T	565.3

Table S5. Results of phenotypic and physiologic tests for *Nitrospirillum* strains proposed as new species and the type strains *N. viridazoti* BR 11140^T and *N. amazonense* BR 11142^T.

Test	Strains							
	BR11142 ^T	BR11140 ^T	BR11865 ^T	BR11163 ^T	BR11164 ^T	BR11828 ^T	BR11622 ^T	BR11752 ^T
<i>Antibiotic suceptility</i>								
Amoxicillin 10	+	+	+	+	+	+	+	-
Ampicillin 10	+	+	+	+	+	+	-	-
Rifampicin 15	+	-	-	+	+	-	-	-
Chloramphenicol 30	-	+	+	+	+	-	-	-
Azithromycin 15	-	-	+	-	+	-	-	-
Kanamycin 30	-	-	-	-	-	-	-	-
Gentamicin 10	-	-	-	-	-	-	-	-
Neomycin 30	-	-	-	-	-	-	-	-
Cephalexin 30	-	+	-	+	+	-	+	-
Penicillin 10	+	+	+	+	+	+	+	+
Tetracycline 30	-	-	+	+	+	-	-	+
Vancomycin 30	-	-	+	-	-	-	+	+
Streptomycin 10	-	-	-	-	-	-	-	-
Erythromycin 25	-	-	-	+	+	-	-	+

<i>Assimilation - API test</i>								
D-Glucose	-	-	-	-	-	-	-	-
L-Arabinose	+	+	+	+	+	+	+	+
D-Mannose	+	+	-	+	+	+	+	+
D-Mannitol	-	+	w	-	w	w	-	w
N-Acetylglucosamine	-	-	w	-	w	w	-	w
D-Maltose	+	+	-	+	+	+	+	+
Potassium gluconate	-	-	w	-	w	w	-	w
Capric acid	-	-	-	-	-	-	-	-
Adipic acid	-	-	w	-	-	w	-	w
Malic acid	-	-	-	-	-	+	-	+
Trisodium citrate	-	-	-	-	-	+	-	-
Phenylacetic acid	-	-	-	-	-	-	-	w
<i>Enzyme activity - API test</i>								
Reduction of nitrates	-	-	-	-	-	+	-	+
Reduction of nitrates to nitrogen	-	-	-	-	-	-	-	-
L-Tryptophan (tryptophanase)	-	-	-	-	-	-	-	-
D-Glucose (glucokinase)	+	+	+	+	+	+	+	+
L-Arginine (arginase)	-	-	-	+	-	+	+	+
Urea (urease)	+	-	+	+	+	+	+	+
Esculin (β -Glucosidase)	+	+	+	+	+	+	+	+
Ferric citrate (citrase)	+	+	+	+	+	+	+	+
Gelatin (bovine origin)	-	-	-	-	-	-	-	-
Nitrophenyl- β -Dgalactopyranoside (β -Galactosidase)	+	+	+	+	+	+	+	+
<i>Other Physiological characteristics</i>								
Cellulase	-	-	-	-	-	-	+	-
Catalase	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+
Calcium Phosphate solubilization	-	-	-	-	-	-	-	-
Aluminum Phosphate solubilization	-	-	-	-	-	-	-	-
Zinc oxide solubilization	-	-	-	-	-	-	-	-
Zinc carbonate solubilization	-	-	-	-	-	-	-	-
Zinc phosphate solubilization	+	+	+	+	+	+	+	+
Indoles Production (μ g/ml) 48h	2.10	9.00	0.26	1.74	17.29	4.89	nd	6.86
Indoles Production (μ g/ml) 72h	7.97	13.99	4.60	5.50	12.79	12.57	8.84	9.05
Siderophore Production Diameter of the halo (cm) - 14 days	1.48	1.55	1.62	1.59	1.55	1.42	Nd	1.50
Siderophore Production Diameter of the halo (cm) - 7 days	1.77	1.86	1.67	1.78	1.70	1.64	nd	1.64

+ - Positive growth; - no growth; w – weak growth

Table S6. Identification of antimicrobial resistance mechanism and related genes identified using “The Genome Annotation Service in BV-BRC employs a k-mer-based approach.

Antimicrobial Resistance Genes		Genomes of the strains								
AMR Mechanism	Genes	BR 11163 ^T	BR 11164 ^T	BR 11752 ^T	BR 11828 ^T	BR 11140 ^T	BR 11142 ^T	BR 11145 ^T	BR 11865 ^T	DSM 22198 ^T
Antibiotic activation enzyme	KatG									
Antibiotic target in susceptible species	Alr, Ddl, dxr, EF-G, EF-Tu, folA, Dfr, folP, gyrA, gyrB, inhA, fabI, Iso-tRNA, kasA, MurA, rho, rpoB, rpoC, S10p, S12p									
Efflux pump conferring antibiotic resistance	EmrAB-OMF, EmrAB-TolC, MacA, MacB, MdtABC-TolC									
Efflux pump conferring antibiotic resistance	EmrAB-OMF, EmrAB-TolC, MdtABC-TolC									
Gene conferring resistance via absence	gidB									
Protein altering cell wall charge conferring antibiotic resistance	GdpD, PgsA									
Protein modulating permeability to antibiotic	OprB family									
Regulator modulating expression of antibiotic resistance genes	OxyR									

Gray indicates positive, and white negative.

Table S7. Identity values of 16S rRNA between strains of the genus *Nitrospirillum*.

Strains	DSM 22198 ^T (<i>N.iris</i>)	BR 11142 ^T (<i>N. amazonense</i>)	BR 11140 ^T (<i>N. viridazoti</i>)	BR 11865 ^T (<i>N. bahiense</i>)	BR 11828 ^T (<i>N. guiasense</i>)	BR 11752 ^T (<i>N. karajorum</i>)	BR 11164 ^T (<i>N. guarani</i>)	BR 11163 ^T (<i>N. guanabarens</i>)	BR 11622 ^T (<i>N. pindoramense</i>)
DSM 22198 ^T (<i>N. iris</i>)	1,00	0,97	0,97	0,97	0,97	0,98	0,97	0,97	0,98
BR 11142 ^T (<i>N. amazonense</i>)	0,97	1,00	0,99	0,99	0,98	0,99	0,99	1,00	0,99
BR 11140 ^T (<i>N. viridazoti</i>)	0,97	0,99	1,00	0,99	0,99	0,98	0,99	0,99	0,99
BR 11865 ^T (<i>N. bahiense</i>)	0,97	0,99	0,99	1,00	0,98	0,99	0,98	0,99	0,99
BR 11997	0,98	1,00	0,99	1,00	0,98	1,00	0,99	0,99	0,99
BR 11994	0,98	0,99	0,99	1,00	0,98	0,99	0,99	0,99	0,99
BR 11868	0,97	0,99	0,99	0,99	0,98	0,99	0,98	0,99	0,98
BR 11858	0,98	0,99	0,99	1,00	0,98	0,99	0,99	0,99	0,99
BR 11845	0,97	0,99	0,99	1,00	0,98	0,99	0,99	0,99	0,99
BR 11838	0,97	0,99	0,99	1,00	0,98	0,99	0,98	0,99	0,98
BR 11645	0,97	1,00	0,99	1,00	0,98	0,99	0,99	0,99	0,99
BR 11644	0,97	1,00	0,99	1,00	0,98	0,99	0,99	1,00	0,99
BR 11695	0,98	0,99	0,99	1,00	0,98	0,99	0,99	0,99	0,99
BR 11828 ^T (<i>N. guiasense</i>)	0,97	0,98	0,99	0,98	1,00	0,99	0,98	0,98	0,99
BR 11938	0,98	0,98	0,99	0,99	1,00	0,99	0,98	0,98	0,99
BR 11870	0,98	0,98	0,99	0,99	1,00	0,99	0,98	0,98	0,99
BR 11855	0,98	0,98	0,99	0,99	1,00	0,99	0,98	0,98	0,99
BR 11755	0,98	0,98	0,99	0,99	1,00	0,99	0,98	0,98	0,99
BR 11754	0,97	0,98	0,99	0,98	1,00	0,99	0,98	0,98	0,99
BR 11736	0,98	0,98	0,99	0,99	1,00	0,99	0,98	0,98	0,99

BR 11739	0,97	0,98	0,99	0,98	0,99	0,99	0,98	0,98	0,99
BR 11746	0,97	0,98	0,99	0,98	0,99	0,99	0,98	0,98	0,99
BR 11747	0,97	0,98	0,99	0,99	1,00	0,99	0,98	0,98	0,99
BR 11752 ^T (<i>N. karajorum</i>)	0,98	0,99	0,98	0,99	0,99	1,00	0,98	0,99	0,99
BR 11737	0,97	0,99	0,98	0,99	0,99	1,00	0,98	0,99	0,99
BR 11742	0,97	0,99	0,98	0,99	0,99	1,00	0,99	0,99	0,99
BR 11745	0,97	0,99	0,98	0,99	0,99	1,00	0,98	0,99	0,99
BR 11832	0,98	0,99	0,98	0,99	0,99	1,00	0,98	0,99	0,99
BR 11872	0,97	0,99	0,98	0,99	0,99	1,00	0,98	0,99	0,99
BR 11850	0,97	0,99	0,98	0,99	0,99	1,00	0,98	0,99	0,99
BR 11853	0,97	0,99	0,98	0,99	0,99	0,99	0,98	0,99	0,98
BR 11869	0,97	0,99	0,98	0,99	0,99	0,99	0,98	0,99	0,98
BR 11926	0,97	0,99	0,98	0,99	0,99	1,00	0,98	0,99	0,99
BR 11928	0,97	0,99	0,98	0,99	0,98	0,99	0,98	0,99	0,99
BR 11935	0,97	0,99	0,98	0,99	0,98	0,99	0,98	0,99	0,98
BR 11164 ^T (<i>N. guarani</i>)	0,97	0,99	0,99	0,98	0,98	0,98	1,00	0,99	0,98
BR 11168	0,97	0,99	0,99	0,99	0,98	0,98	1,00	0,99	0,98
BR 11170	0,97	0,99	0,99	0,99	0,98	0,98	1,00	0,99	0,98
BR 11640	0,97	0,99	0,99	0,99	0,98	0,98	1,00	0,99	0,98
BR 11163 ^T (<i>N. guanabarensense</i>)	0,97	1,00	0,99	0,99	0,98	0,99	0,99	1,00	0,98
BR 11162	0,97	1,00	0,99	0,99	0,98	0,99	0,99	1,00	0,98
BR 11169	0,97	0,99	0,99	0,99	0,98	0,99	0,98	1,00	0,98
BR 11622 ^T (<i>N. pindoramense</i>)	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11991	0,97	0,98	0,99	0,98	0,99	0,99	0,98	0,98	0,99

BR 11967	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,98	0,99
BR 11966	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,98	0,99
BR 11949	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,98	1,00
BR 11948	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11945	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11944	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11943	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11937	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11933	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11877	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11876	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,98	1,00
BR 11875	0,97	0,98	0,99	0,98	0,99	0,99	0,98	0,98	1,00
BR 11874	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11857	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11852	0,98	0,99	0,98	0,99	0,99	1,00	0,98	0,99	0,99
BR 11851	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11835	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11834	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11833	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11830	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11829	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11873	0,97	0,99	0,98	0,99	0,98	0,99	0,98	0,99	0,99
BR 11751	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11750	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00
BR 11748	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11743	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,99	1,00

BR 11741	0,97	0,98	0,99	0,98	0,99	0,98	0,98	0,98	0,99
BR 11740	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11639	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11637	0,97	0,98	0,99	0,98	0,99	0,99	0,98	0,98	0,99
BR 11636	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11635	0,97	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11634	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11633	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11632	0,97	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11630	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11629	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11628	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11627	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11625	0,97	0,99	0,99	0,98	0,99	0,99	0,98	0,98	1,00
BR 11624	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11623	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,99	1,00
BR 11621	0,98	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00
BR 11620	0,97	0,98	0,99	0,98	0,99	0,99	0,98	0,98	0,99
BR 11619	0,97	0,99	0,99	0,99	0,99	0,99	0,98	0,98	1,00

Table S8. Fatty acids profiles obtained with the MIDI protocol system with FAME RTSBA6 database of *Nitrospirillum* strains for grown on R2A medium, at 30 °C for 48 h. Analyses performed in a GC Agilent model 7890B GC. The values represent the mean $\pm$ standard deviation of the total percentage of cellular fatty acid composition, based on two replicates (n = 2).

Peak Name	BR 11140 ^T (%)	BR 11163 ^T (%)	BR 11164 ^T (%)	BR 11622 ^T (%)	BR 11752 ^T (%)	BR 11828 ^T (%)	BR 11865 ^T (%)
C _{10:0}	3.2 $\pm$ 0.3	4.5 $\pm$ 0.1	4.6 $\pm$ 0.1	4.9 $\pm$ 0.8	4.4 $\pm$ 0.0	4.4 $\pm$ 0.1	3.4 $\pm$ 0.4
C _{10:0} 3-OH	–	Trace	–	Trace	–	Trace	–
C _{12:0}	Trace	Trace	Trace	Trace	Trace	Trace	Trace
C _{14:0}	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Sum In Feature 1*	Trace	Trace	–	Trace	Trace	–	Trace
C _{15:1} ω 5c	Trace	–	–	–	–	–	–
C _{14:0} 2-OH	3.4 $\pm$ 0.1	3.8 $\pm$ 0.2	3.0 $\pm$ 0.2	3.0 $\pm$ 0.1	1.8 $\pm$ 0.0	2.6 $\pm$ 0.0	3.7 $\pm$ 0.2
Sum In Feature 2*	2.1 $\pm$ 0.1	2.4 $\pm$ 0.1	2.2 $\pm$ 0.1	1.7 $\pm$ 0.2	2.3 $\pm$ 0.1	1.9 $\pm$ 0.1	2.6 $\pm$ 0.0
C _{16:1} ω 9c	Trace	Trace	Trace	–	–	–	–
Sum In Feature 3*	1.9 $\pm$ 0.1	2.1 $\pm$ 0.2	2.2 $\pm$ 0.1	4.8 $\pm$ 0.1	4.1 $\pm$ 0.0	4.5 $\pm$ 0.0	1.6 $\pm$ 0.1
C _{16:1} ω 5c	7.0 $\pm$ 1.2	5.1 $\pm$ 0.4	4.2 $\pm$ 0.0	4.5 $\pm$ 0.1	5.1 $\pm$ 0.3	4.0 $\pm$ 0.3	11.3 $\pm$ 0.7
C _{16:0}	8.3 $\pm$ 0.6	10.3 $\pm$ 0.3	11.6 $\pm$ 0.1	9.3 $\pm$ 0.0	10.8 $\pm$ 0.5	10.9 $\pm$ 0.2	3.1 $\pm$ 0.1
Iso-C _{17:1} ω 5c	Trace	–	–	–	–	–	–
C _{15:0} 3-OH	Trace	–	–	–	–	–	–
C _{17:1} ω 8c	Trace	Trace	Trace	Trace	Trace	Trace	Trace
C _{17:1} ω 6c	Trace	Trace	Trace	Trace	Trace	Trace	Trace
C _{17:0}	Trace	Trace	Trace	Trace	Trace	–	–
C _{16:1} 2-OH	–	Trace	–	Trace	Trace	Trace	–
Iso-C _{16:0} 3-OH	Trace	Trace	Trace	Trace	Trace	Trace	Trace
C _{16:0} 2-OH	1.2 $\pm$ 0.1	1.1 $\pm$ 0.0	Trace	1.1 $\pm$ 0.0	1.3 $\pm$ 0.0	1.1 $\pm$ 0.0	Trace
C _{16:0} 3-OH	8.8 $\pm$ 1.3	5.2 $\pm$ 1.0	5.4 $\pm$ 0.0	4.2 $\pm$ 0.8	4.5 $\pm$ 0.5	4.2 $\pm$ 0.3	10.3 $\pm$ 2.1
Sum In Feature 8*	41.7 $\pm$ 2.1	44.6 $\pm$ 1.1	45.5 $\pm$ 0.2	43.8 $\pm$ 0.8	43.5 $\pm$ 0.5	43.7 $\pm$ 0.2	40.2 $\pm$ 2.2
C _{18:1} ω 5c	Trace	Trace	Trace	Trace	Trace	Trace	Trace
C _{18:0}	Trace	Trace	Trace	Trace	Trace	Trace	Trace
C _{18:1} ω 7c 11-methyl	–	–	Trace	–	Trace	Trace	Trace
Sum In Feature 7*	–	–	Trace	Trace	Trace	Trace	–
C _{19:0} cyclo ω 8c	Trace	1.5 $\pm$ 0.1	2.0 $\pm$ 0.0	1.1 $\pm$ 0.3	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	Trace

C _{18:1} 2-OH	20.1 ± 0.1	17.5 ± 0.3	16.5 ± 0.1	19.9 ± 0.6	19.3 ± 0.3	19.3 ± 0.0	21.0 ± 0.1
C _{20:4} ω6,9,12,15c	–	–	Trace	–	–	–	–
C _{18:0} 3-OH	–	–	–	–	–	–	Trace
Summed Feature 1*	Trace	Trace	–	Trace	Trace	–	Trace
Summed Feature 2*	2.1 ± 0.1	2.4 ± 0.1	2.2 ± 0.1	1.7 ± 0.2	2.3 ± 0.1	1.9 ± 0.1	2.6 ± 0.0
Summed Feature 3*	1.9 ± 0.1	2.1 ± 0.2	2.2 ± 0.1	4.8 ± 0.1	4.1 ± 0.0	4.5 ± 0.0	1.6 ± 0.1
Summed Feature 7*	–	–	Trace	Trace	Trace	Trace	–
Summed Feature 8*	41.7 ± 2.1	44.6 ± 1.1	45.5 ± 0.2	43.8 ± 0.8	43.5 ± 0.5	43.7 ± 0.2	40.2 ± 2.2

Trace, Trace amount (<1%); –, Not detected. *Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The Sherlock® Microbial Identification System version 6.5 (MIDI) using the RTSBA6 database groups these fatty acids together as one feature with a single percentage of the total. Summed Feature 1 comprises Iso-C_{15:1} H and/or C_{13:0} 3-OH, Summed Feature 2 comprises C_{14:0} 3-OH and/or Iso-C_{16:1} I, Summed Feature 3 comprises C_{16:1} ω7c and/or C_{16:1} ω6c, Summed Feature 7 comprises C_{19:1} ω6c and/or C_{19:1} ω7c and/or C_{19:1} 19cy and Summed Feature 8 comprises C_{18:1} ω7c and/or C_{18:1} ω6c.

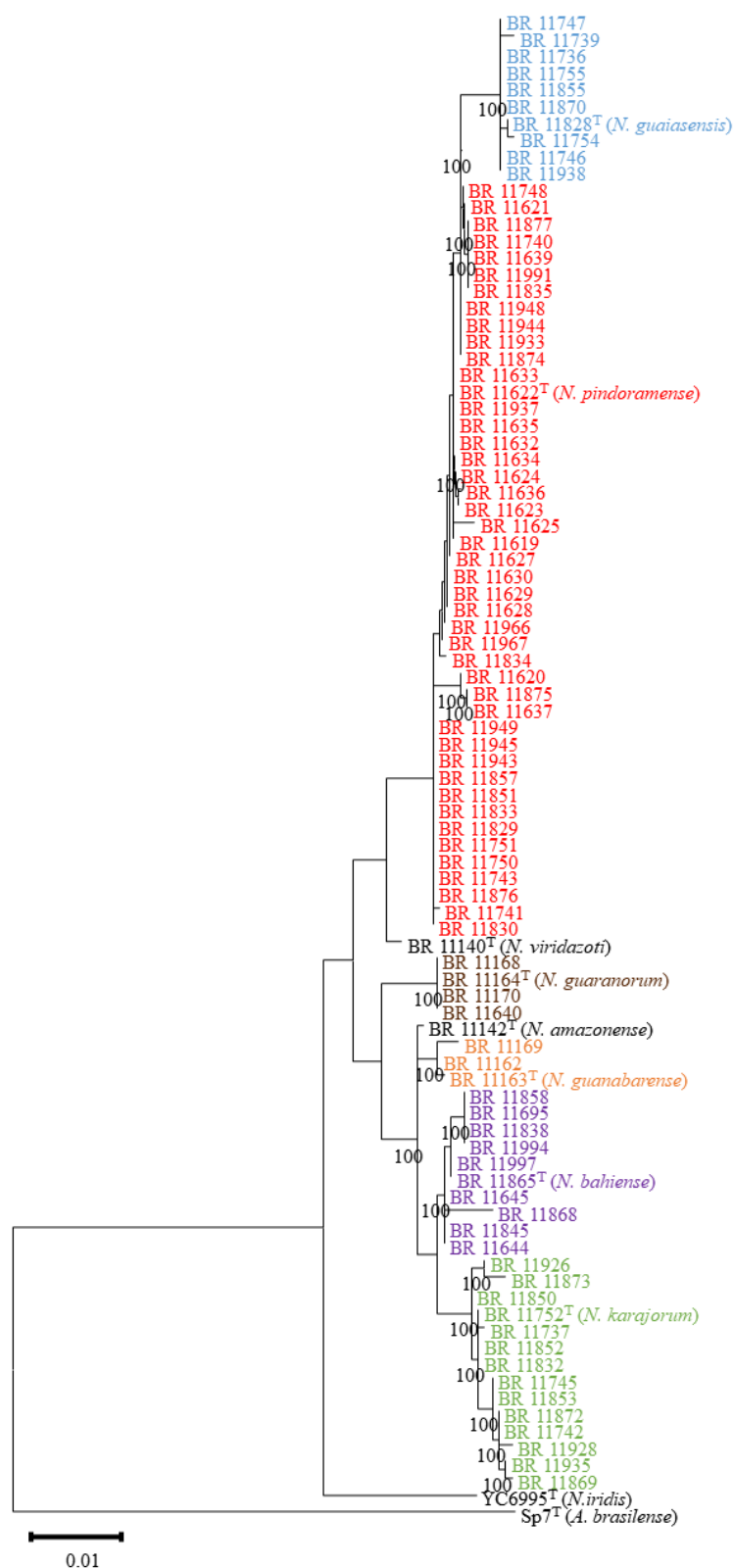


Fig. S1. Phylogenetic tree based on the 16S rRNA of *Nitrospirillum* strains proposed as new species and the type strains *N. iridis* DSM 22198^T, *N. viridazoti* BR 11140^T and *N. amazonense* BR 11142^T. Bootstrap values, 500 repetition, are shown.

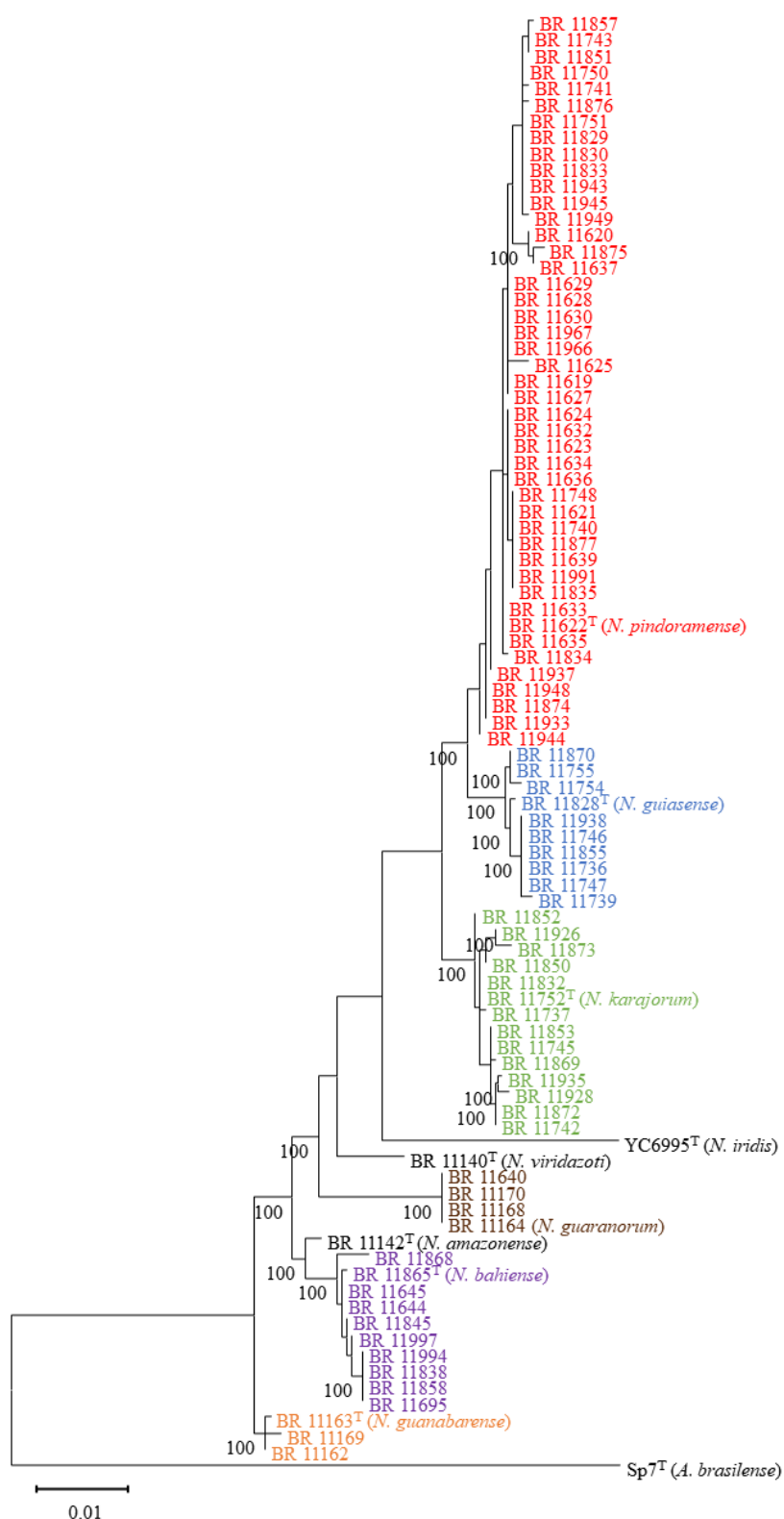
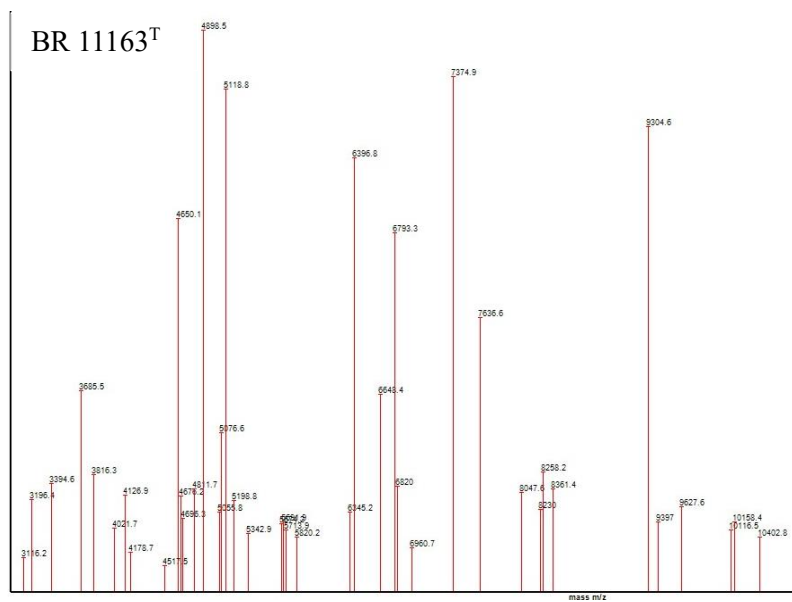
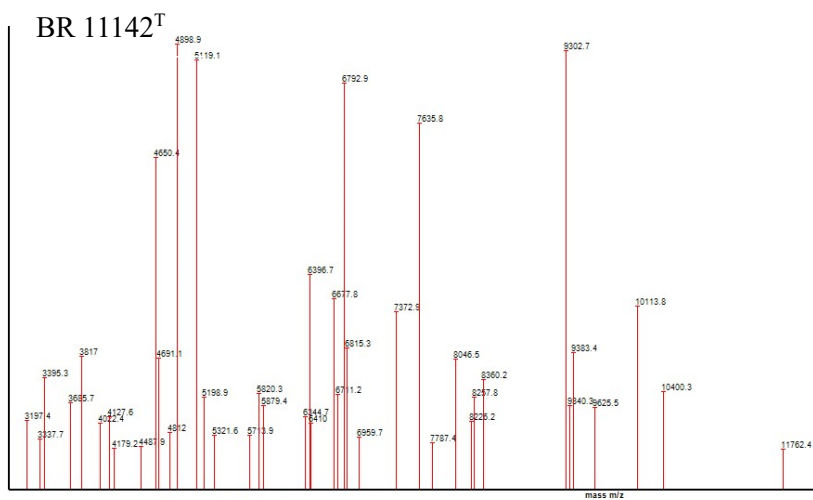
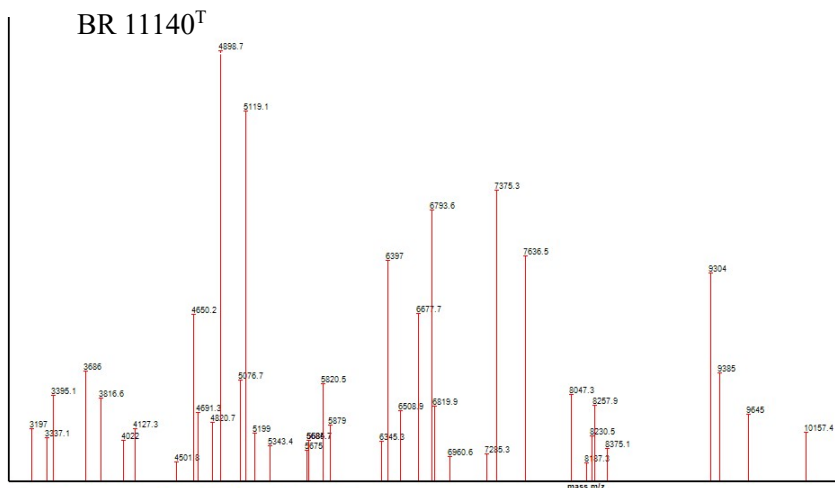
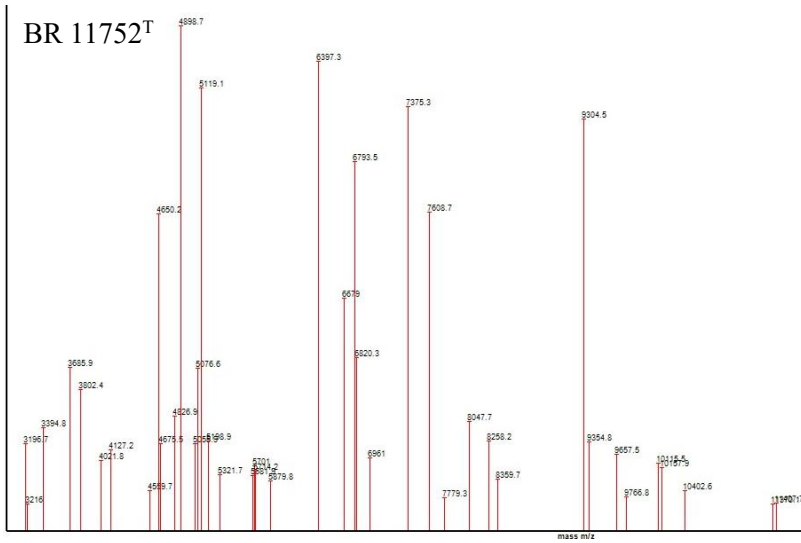
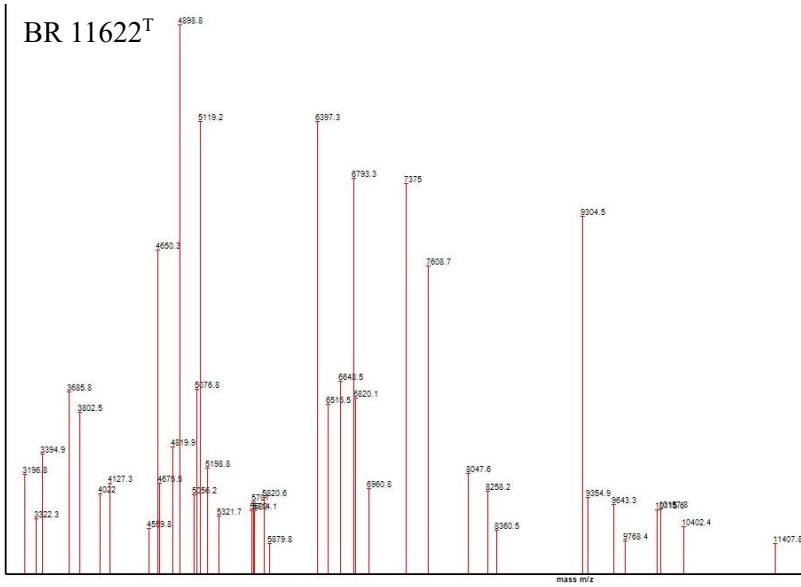
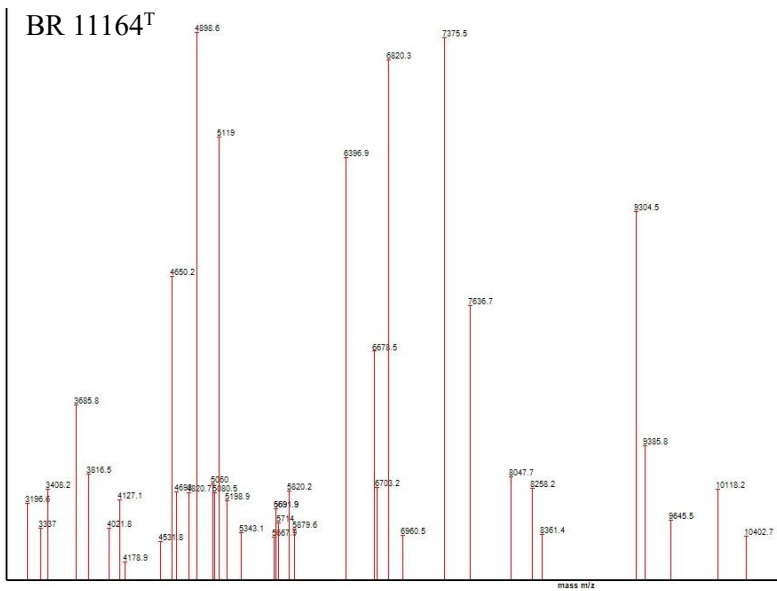


Fig. S2. Phylogenetic tree based on the *recA* gene of *Nitrospirillum* strains proposed as new species and the type strains *N. iridis* DSM 22198^T, *N. viridazoti* BR 11140^T and *N. amazonense* BR 11142^T. Bootstrap values, 500 repetition, are shown.





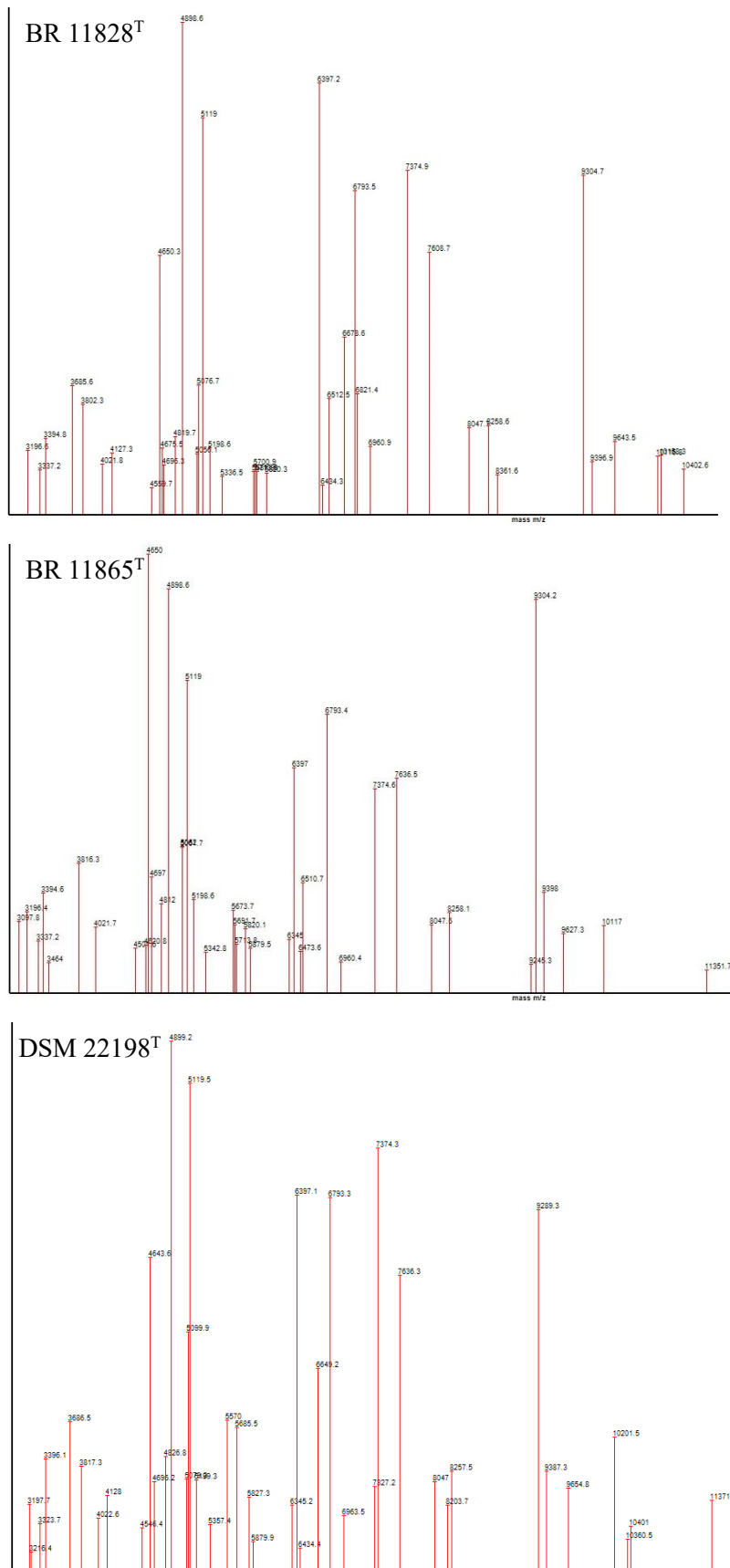


Fig. S3. MALDI-TOF MS SuperSpectra of *Nitrospirillum* strains proposed as new species and the type strains *N. iridis* DSM 22198^T, *N. viridazoti* BR 11140^T and *N. amazonense* BR 11142^T generated by SARAMIS package with multiple individual spectra of whole cell protein of bacterial grown in different culture media and different time.

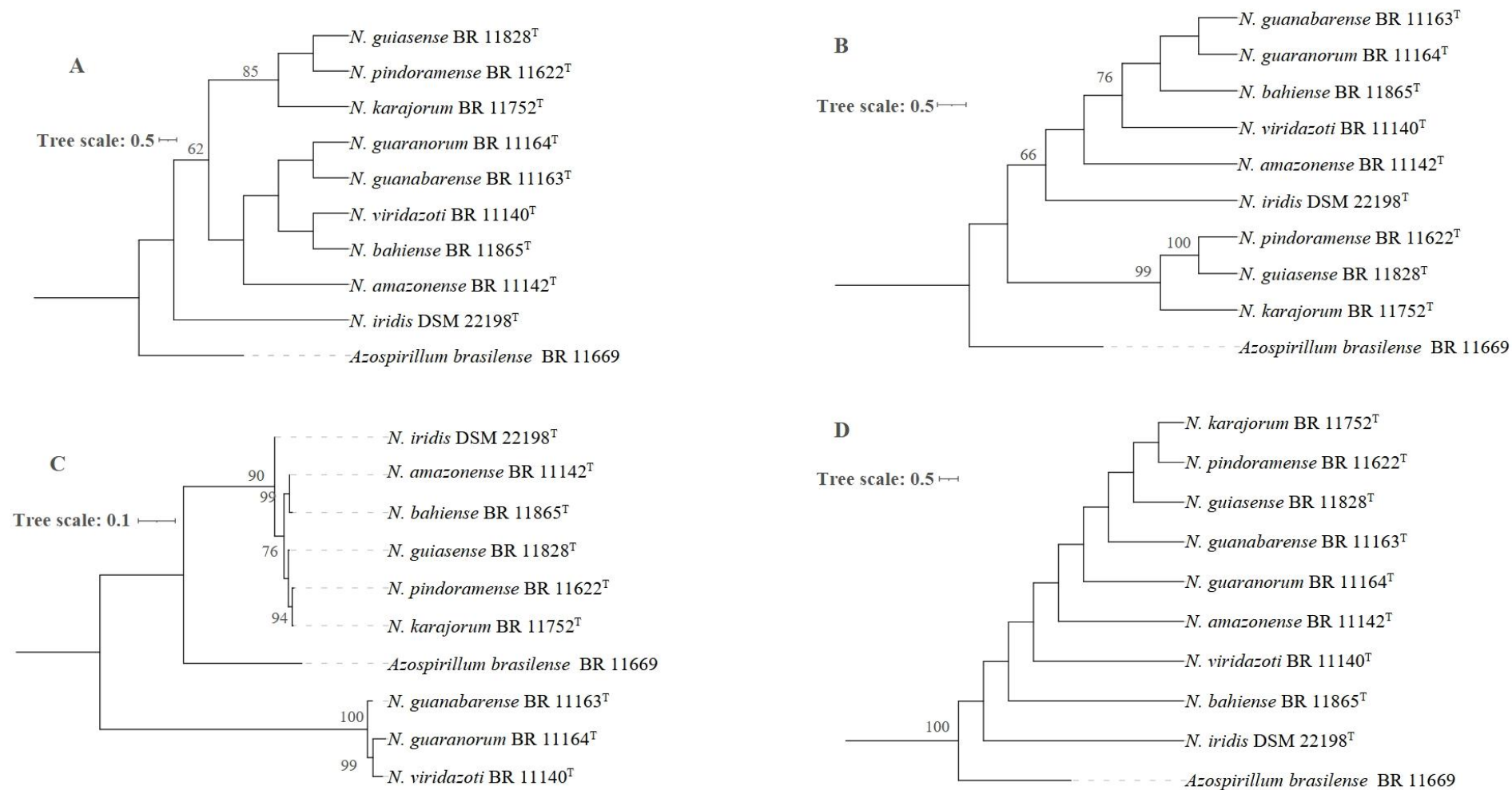


Fig. S4 Phylogenetic tree conducted from the protein sequences of the respective genes: (A) Dps gene (decaprenyl diphosphate synthase), (B) operon containing fadN-fadA-fadE genes, (C) PlsX gene (acyl-ACP phosphate acyltransferase and (D) YciA gene (Acyl-CoA thioesterase). The evolutionary history was inferred using the Maximum Likelihood method, based on the evolutionary model of Tamura-Nei. Bootstrap values above 50% with 500 repetitions are shown.

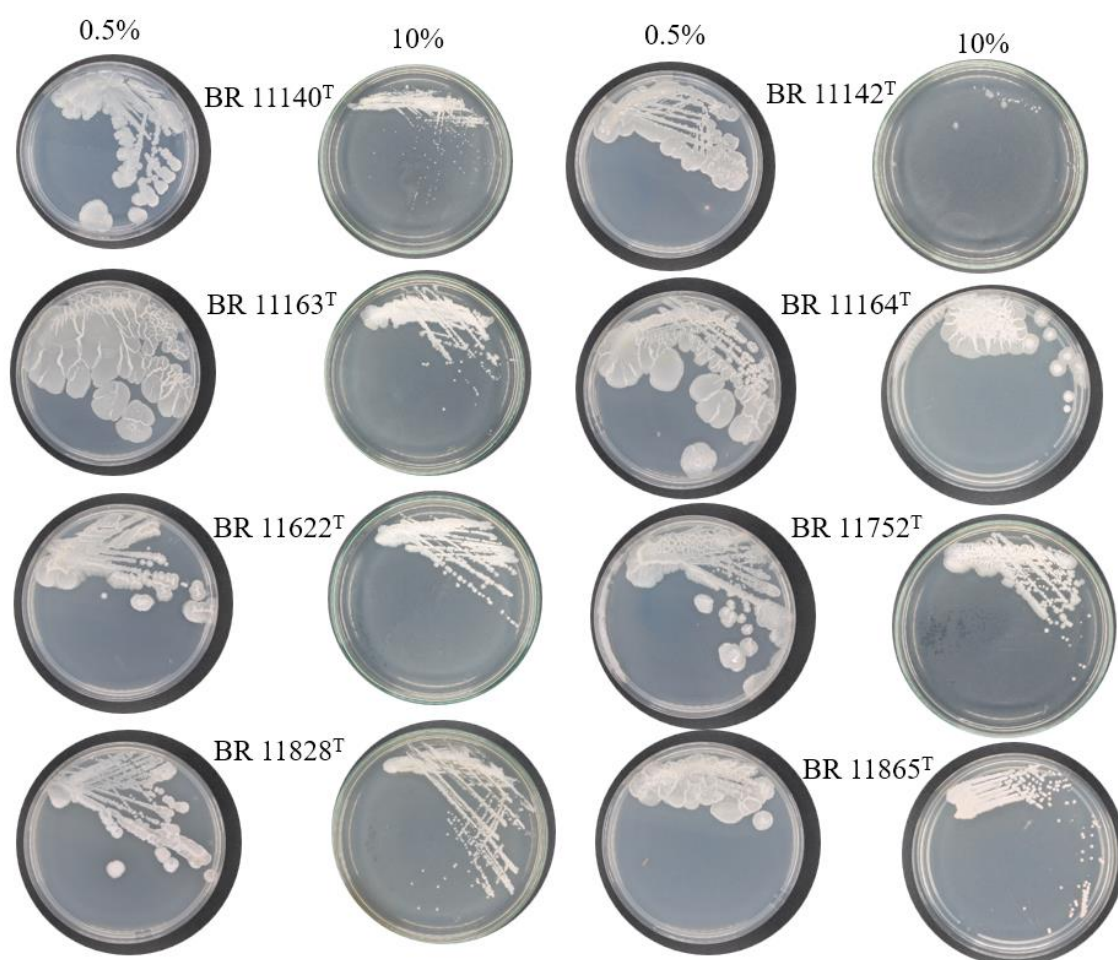


Fig. S5. Details of the growth of *Nitrospirillum* spp. strains on plates containing homemade potato agar medium supplemented with sucrose.

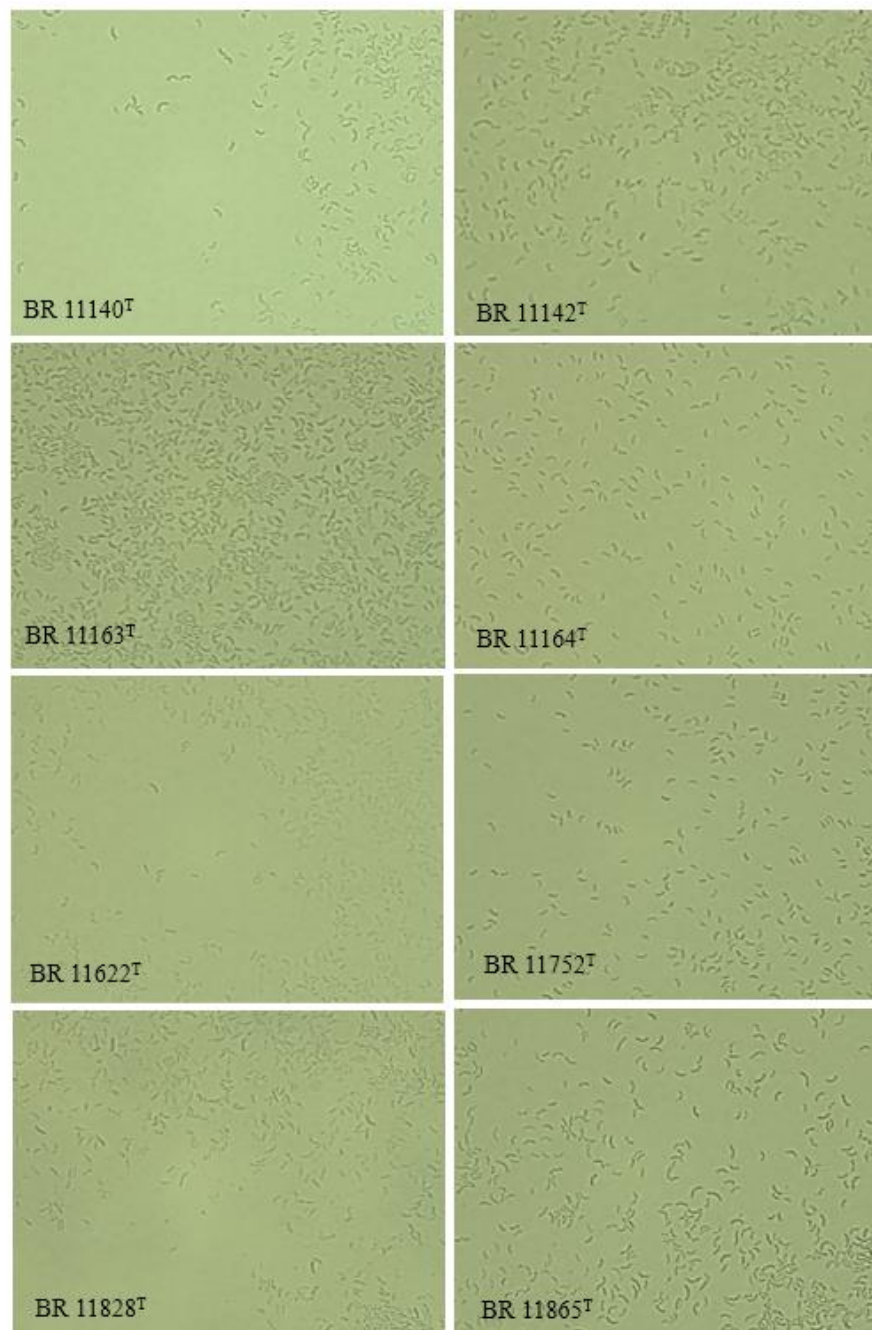


Fig. S6. Details of the cell morphology of *Nitrospirillum* spp. strains grown in nitrogen-free LGI semi-solid medium.



5 days



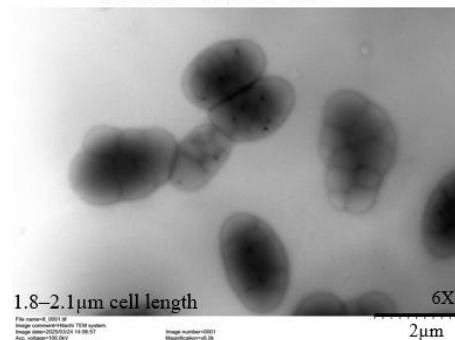
10 days



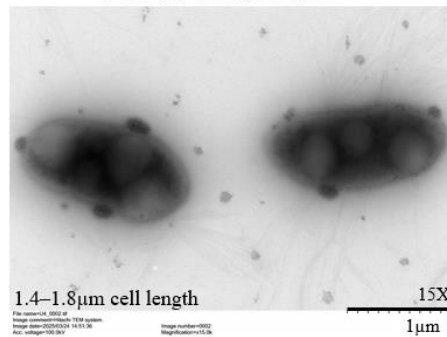
15 days

Fig. S7. Details of the pellicle formation over the nitrogen-free LGI semi-solid medium of *Nitrospirillum* spp. strains. From the left to right: BR 11140^T and BR 11142^T BR 11163^T, BR 11164^T, BR 11622^T, BR 11752^T, BR 11828^T and BR 11865^T

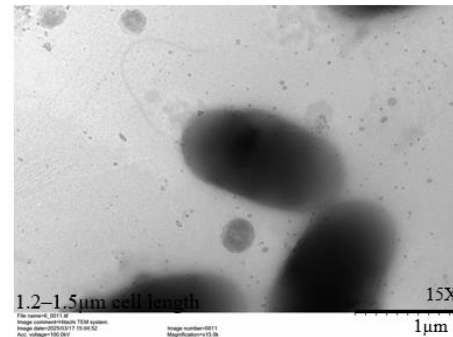
BR 11140^T



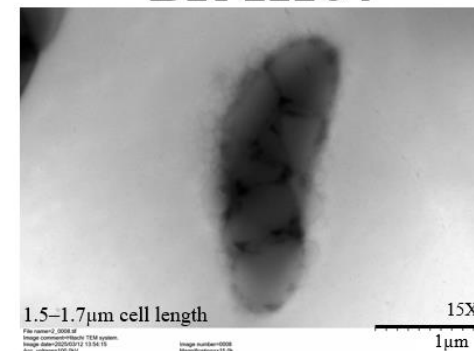
BR 11142^T



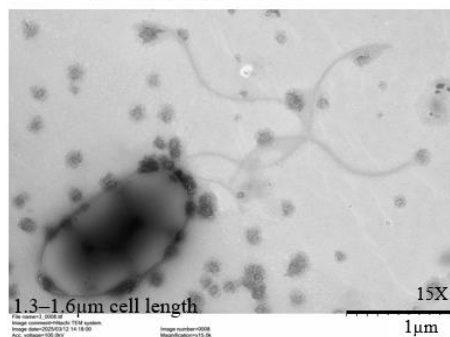
BR 11163^T



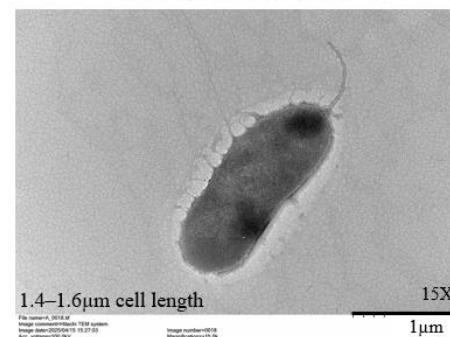
BR 11164^T



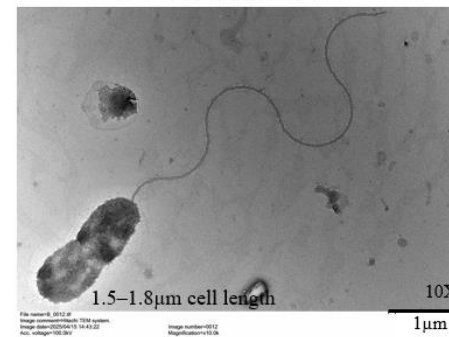
BR 11622^T



BR 111752^T



BR 11828^T



BR 11865^T

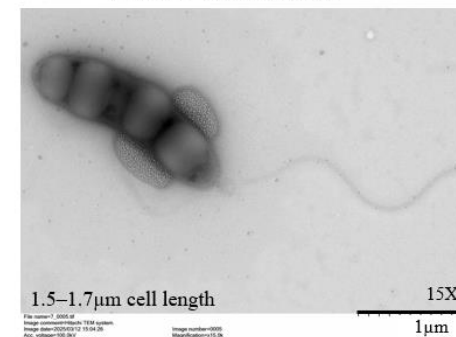


Fig. S8. Image of strain the *Nitrospirillum* spp. obtained by transmission electron microscopy. The magnification and cells sizes are presented in the figures.

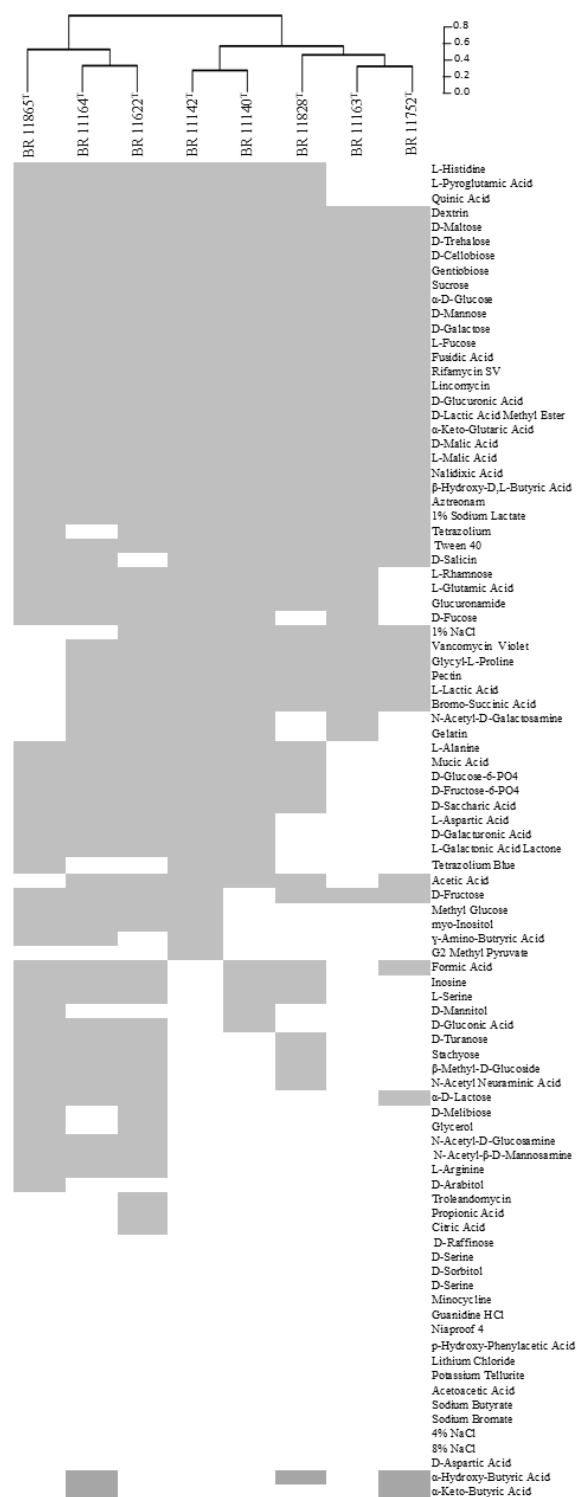


Fig. S9. Dendrogram of *Nitrospirillum* spp. constructed with the Biolog GN III test and heatmap showing the positive (gray square) and negative reaction (blank square) for *N. amazonense* strain BR 11142^T, *N. azotoviridis* strain BR 11140^T and the proposed new species.