

Supplementary material

Table S1: Genome characteristics of the five *Bradyrhizobium* sp. strains

Description	<i>Bradyrhizobium</i> sp. BA2	<i>Bradyrhizobium</i> sp. RDT10	<i>Bradyrhizobium</i> sp. RDT46	<i>Bradyrhizobium</i> sp. RDI18	<i>Bradyrhizobium</i> sp. TZ2
GenBank accession number	GCA_042691665.1	GCA_044772375.1	GCA_042019905.1	GCA_043203335.1	GCA_043099345.1
Average coverage depth	64.0x	48.0x	23.0x	27.0x	37.0x
Contig count	2	1	1	9	1
Largest contig (bp)	7966224	8987948	6966653	7032965	7954720
Total length (bp)	8461354	8987948	6966653	8864934	7954720
Contigs N ₅₀ (bp)	7966224	8987948	6966653	7032965	7954720
Contigs L ₅₀	1	1	1	1	1
Total number of genes	8 100	9346	6 654	8 659	8 065
Total coding sequences (CDSs)	7 532	6920	5854	7 576	6 123
Number of tRNA	48	52	48	51	49
G+C content (mol%)	63.07	62.09	64.77	61.96	61.7

Table S2: Genes involved in nodulation process predicted for *Bradyrhizobium* sp. strains BA2, RDT10, RDT46, RDI18 and TZ2

	Gene name	Gene annotation	RDT46		BA2		RDI18		TZ2		RDT10		<i>B. Japonicum</i>		<i>B. elkani</i>	
			Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
Nodulation genes	<i>nodV</i>	Nodulation protein NodV	-	-	-	-	-	-	-	-	1022081	1022719	-	-	-	-
	<i>nodO</i>	symbiont process	-	-	5841996	5840284	2247380	2246889	3888591	3890321	5046817	5048862	8086424	8084817	-	-
	<i>nodA</i>	hydroxyacid dehydrogenase	-	-	5848502	5848726	2254484	2255191	3878125	3878820	5061197	5061904	8099039	8099683	7127814	7128464
	<i>nodU</i>	nodulation protein NodU	-	-	5763876	5764529	2151341	2151979	3673923	3674477	4948004	4948642	8289068	8289706	7501441	7502073
	<i>nodB</i>	nodulation protein NodB	-	-	5765397	5765888	2152862	2153482	3672439	3672955	1036878	1037426	1786169	1786669	7502961	7503479
	<i>nodW</i>	nodulation protein NodW	-	-	5766096	5766728	-	-	3671536	3672237	1037733	1038425	-	-	7503651	7504346
	<i>nodL</i>	nodulation factor fucose acetyltransferase NodL	-	-	5811362	5812483	2844896	2846008	3942300	3943421	5668291	5669217	-	-	-	-
	<i>nodZ</i>	nodulation protein NodZ	-	-	5835204	5836178	2844896	2846008	3894785	3895771	5045432	5046421	1992976	1994088	7113045	7114157
	<i>nodI</i>	nodulation factor ABC transporter ATP-binding protein NodI	-	-	5839365	5840279	2239926	2240915	3890309	3891223	5049748	5050662	1988816	1989736	7117427	7118347
	<i>nodU</i>	nodulation protein NodU	-	-	5840284	5841996	2244243	2245157	3888591	3890299	5050672	5052381	1987105	1988814	7436813	7438471
	<i>nodS</i>	nodulation methyltransferase NodS	-	-	5842008	5842637	2245167	2246876	3887948	3888547	5052394	5053011	1986462	1987091	-	-
	<i>nodC</i>	chitooligosaccharide synthase NodC	-	-	5842662	5844017	2174788	2175387	3886562	3887917	5053052	5054416	8092549	8091092	7120716	7122089
	<i>nodB</i>	chitooligosaccharide deacetylase NodB	-	-	5844032	5844691	2247548	2248912	3885887	3886546	5054433	5055092	8093223	8092564	7122106	7122765
	<i>nodA</i>	NodA family N-acetyltransferase	-	-	5844688	5845320	2248929	2249588	3884323	3884787	5055089	5055721	8093852	8093220	7122762	7123394
	<i>nodD2</i>	Nodulation protein D 2	3389461	3390393	5844458	5845402	2250211	2252902	3880913	3883485	5056526	5059227	8097059	8098051	7125830	7126825
	<i>nodT</i>	Nodulation protein NodT	-	-	3425341	3426192	2151990	2152856	3672057	3672806	4948653	4949243	8289718	8290581	7502085	7502960
	<i>NfeD</i>	nodulation protein NfeD	-	-	380646	381092	2977562	2978959	-	-	5677272	5678670	-	-	8010362	8010808
	<i>nodF</i>	nodulation protein F	-	-	-	-	-	-	3718816	3718995	-	-	-	-	-	-
	<i>nodJ</i>	Part of the ABC transporter complex NodJ involved in the exp	-	-	-	-	-	-	3891220	3892017	5048956	5049744	-	-	-	-
	<i>nodD1</i>	LysR substrate binding domain	-	-	-	-	-	-	-	-	5056526	5059227	8095589	8096425	7124245	7125189

Table S3: Genes involved in Type III secretion system predicted for *Bradyrhizobium* sp. strains BA2, RDT10, RDT46, RDI18 and TZ2

	Gene name	Gene annotation	RDT46		BA2		RDI18		TZ2		RDT10		<i>B. Japonicum</i>		<i>B. elkani</i>	
			Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
T3SS complex	<i>scfT</i>	type III secretion system export apparatus subunit ScfT	-	-	5758371	5759186	2146814	2145993	3679164	3679979	5357504	5358323	8284522	8283701	7496895	7496074
	<i>scfR</i>	type III secretion system export apparatus subunit ScfR	-	-	5759622	5760287	2147102	2147767	3678064	3678729	5358611	5359276	8285476	8284611	7497849	7497184
	<i>scfQ</i>	type III secretion system cytoplasmic ring protein ScfQ	-	-	5760280	5761392	2147760	2148815	3677571	3678071	5359269	5360324	8286590	8285469	7498957	7497842
	<i>scfV</i>	protein secretion by the type III secretion system	-	-	5761904	5763259	2149381	2150731	3675142	3676448	5360888	5362237	8288454	8287099	7500824	7499469
	<i>scfL</i>	type III secretion system stator protein ScfL	-	-	5763256	5763879	2150728	2151351	3674474	3675097	5362239	5362855	8289071	8288451	7501444	7500821
	<i>scfJ</i>	type III secretion system inner membrane ring lipoprotein ScfJ	-	-	5764536	5765387	2151990	2152865	3672965	3673834	5363494	5364344	8290581	8289718	7502960	7502085
	<i>scfV</i>	type III secretion system export apparatus subunit ScfV	-	-	5773256	5775355	2167664	2169817	3662565	3664683	5376876	5378992	8300034	8302133	7519319	7521442
	<i>scfS</i>	EscS/YscS/HrcS family type III secretion system	-	-	5759345	5759620	2147099	2146815	3678731	3679006	5358324	5358608	8284808	8284533	7497181	7496906
	<i>scfU</i>	EscU/YscU/HrcU family type III secretion system export apparatus switch protein	695054	695317	5757337	5758374	2144958	2145996	3679976	3681011	5356470	5357507	8283704	8282667	7496077	7495040
	<i>scfC1</i>	Type III secretion system secretin	4982888	4984384	5753530	5754834	2142894	2144243	3670628	3671299	5354828	5355754	8291273	8291965	7503651	7504346
	<i>scfC2</i>	Type III secretion system secretin	5871547	5873037	5764392	5765024	2153563	2154261	3680932	3682365	5365067	5365771	8262784	8264238	7512639	7512887
	<i>flpP</i>	flagellar type III secretion system pore protein "FlpP"	235764	236522	2235536	2236297	776721	777477	6723934	6724688	1674612	1674959	2600483	2601223	3667072	3666398
	<i>xopAG</i>	XopAG/AwGf1 family type III secretion system effector	-	-	6155285	6156577	2304093	2305481	6373485	6374771	5511088	5512524	-	-	-	-
	<i>xopH</i>	type III secretion system effector XopH	-	-	-	-	2348547	2349803	-	-	6350248	6351504	-	-	-	-
	<i>nopX</i>	hypothetical protein	-	-	3420963	3422735	-	-	3667330	3667635	-	-	-	-	-	-
	<i>nopA</i>	nodulation protein NopA	-	-	5771010	5771243	-	-	-	-	-	-	-	-	-	-
	<i>nopL</i>	secretin N-terminal domain-containing protein	-	-	-	-	2154363	2154605	-	-	-	-	-	-	-	-
	<i>nopP</i>	Effector protein NopP	-	-	5833667	5834251	2178565	2179425	3659690	3660121	5345199	5345372	-	-	7022940	7023761

Table S4: Genes involved in Nitrogen fixation predicted for *Bradyrhizobium* sp. strains BA2, RDT10, RDT46, RDI18 and TZ2

	Gene name	Gene annotation	RDT46		BA2		RDI18		TZ2		RDT10		<i>B. Japonicum</i>		<i>B. elkanii</i>	
			Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
Nitrogen fixation genes	<i>nifA</i>	nif-specific transcriptional activator NifA	556325	558060	5829456	5831279	2230055	2231809	3897862	3899616	5452827	5453945	1995906	1997654	7109382	7111205
	<i>nifD</i>	nitrogenase molybdenum-iron protein alpha chain	564483	565985	6227114	6228616	574195	575699	3620476	3621976	6145056	6146501	1730592	1732094	7581983	7583485
	<i>nifK</i>	nitrogenase molybdenum-iron protein subunit beta	560688	567627	6225474	6227033	572565	574124	3622049	3623609	6143427	6144985	1732160	1733716	7580339	7581895
	<i>nifB</i>	nitrogenase cofactor biosynthesis protein Nif	577452	579013	6203524	6205073	548981	550480	3638378	3639940	6112854	6114353	1744626	1746125	7567621	7569177
	<i>nifH</i>	nitrogenase iron protein	586266	589155	6141322	6142212	2636454	2837338	3700704	3701588	6072828	6073694	1751334	1752218	7558351	7559235
	<i>nifW</i>	nitrogenase stabilizing/protective protein NifW	592530	592859	6139860	6140212	2833195	2833536	3702558	3702899	6069566	6069907	1753796	1754137	7555402	7555743
	<i>nifS</i>	cysteine desulfurase NifS	574873	576000	6208387	6209580	551901	553094	3635818	3637017	2241552	2242736	1742476	1743657	7570593	7571798
	<i>nifZ</i>	nitrogen fixation protein NifZ	580872	581428	6201728	6202299	546543	547099	3640374	3640948	6110419	6110658	1746611	1746925	7566314	7566870
	<i>nifE</i>	nitrogenase iron-molybdenum cofactor biosynthesis protein NifE	567744	569411	6223713	6225356	570802	572466	3623704	3625377	6142059	6143140	1733809	1735452	7578596	7580242
	<i>nifN</i>	nitrogenase iron-molybdenum cofactor biosynthesis protein NifN	569421	570804	6222297	6223703	569386	570792	3625387	3626700	6140250	6141656	1735462	1736871	7577186	7578586
	<i>nifX</i>	nitrogen fixation protein NifX	570826	571220	6221905	6222300	568994	569389	3626790	3627184	6139858	6140253	1736868	1737263	7576794	7577189
	<i>nifT</i>	putative nitrogen fixation protein NifT	576066	576291	6208169	6208390	551680	551904	3637022	3637246	6115554	6115777	1743654	1743878	7570372	7570596
	<i>nifQ</i>	nitrogen fixation protein NifQ	589242	589937	6140491	6141255	2835615	2836325	3701626	3702441	6071983	6072699	1752323	1753141	7557501	7558118
	<i>nifV</i>	homocitrate synthase	590557	591738	6205699	6206892	2833728	2834916	3701716	3702441	6070129	6071291	-	-	7555935	7557122
	<i>nifU</i>	NifU family protein	574550	574843	6209892	6209999	3147747	3148316	3635532	3635821	6116978	6117268	38716	39285	195452	196021
	<i>fixX</i>	glucose 1-dehydrogenase	1021645	1021941	3054693	3054989	2828415	2828711	3704929	3705225	2191561	2191857	8319408	8319704	7551652	7551948
	<i>fixK</i>	Rieske (2Fe-2S) protein	1130352	1131065	6648115	6648801	715722	716417	3588267	3589040	2417308	2418027	7991408	7992091	7005536	7006219
	<i>fixL</i>	sensor protein FixL	943599	944015	6000259	6000594	712996	714500	3586409	3587944	2419923	2421458	2864642	2866159	7001524	7002954
	<i>fixP</i>	cytochrome-c oxidase	961385	962260	6149319	6150035	706559	707428	3592507	3592863	2413484	2414353	7204086	7204961	6995438	6996310
	<i>fixN</i>	GcrA family cell cycle regulator	958805	960358	6146740	6148389	708357	710006	3589417	3591066	2415282	2416931	7205891	7207582	6997236	6998888
	<i>fixJ</i>	response regulator FixJ	863656	864273	5046747	5047364	714521	715137	3585802	3586419	2421448	2422065	2864631	2864638	7002951	7003556
	<i>fixJ</i>	2Fe-2S iron-sulfur cluster-binding protein	1034668	1034976	6425738	6426208	555014	554607	3701626	3702441	2233741	223392	1748635	1748982	-	-
	<i>fixX</i>	(2Fe-2S) ferredoxin domain-containing protein	587006	587359	6672897	6673310	541130	541477	6803766	6803644	2244674	2244264	2834379	2834828	7562195	7562542
	<i>fixA</i>	ferredoxin FdxA	976284	976622	7810376	7810714	3020556	3020894	3478364	3478702	8530999	8531337	724182	724520	474131	474469
	<i>fixB</i>	ferredoxin III	571926	572210	6220918	6221202	567892	568291	3627884	3628168	6138856	6138915	1737959	1738252	7575795	7576094
	<i>fixE</i>	2Fe-2S iron-sulfur cluster-binding protein	1049250	1049570	7023061	7023381	838413	838733	4001904	4002224	6728518	6728838	3848928	3849968	3551554	3551874

Table S5: Genes involved in Abiotic stress response predicted for *Bradyrhizobium* sp. strains BA2, RDT10, RDT46, RDI18 and TZ2 (each number indicates the number of genes copy)

Gene name	RDT46	RDI18	BA2	TZ2	RDT10	Protein product	Gene functionality
<i>putA</i>	1	1	1	1	1	proline dehydrogenase/oxidoreductase/regulation of DNA-templated	General Metabolic Adjustments
<i>betA</i>	1	0	1	0	1	Genes involved in the synthesis of glycine betaine from choline	
<i>betB</i>	0	1	0	0	1	Genes involved in the synthesis of glycine betaine from choline	
<i>proA</i>	1	0	3	4	0	Genes involved in proline biosynthesis	
<i>proB</i>	1	1	1	1	1	Genes involved in proline biosynthesis	
<i>proC</i>	1	1	1	1	1	Genes involved in proline biosynthesis	
<i>proS</i>	1	1	1	1	1	proline-tRNA ligase	
<i>otsA</i>	1	1	1	0	1	Genes for trehalose biosynthesis	
<i>otsB</i>	1	1	1	1	1	Genes for trehalose biosynthesis	
<i>treY</i>	1	1	1	1	1	Additional genes involved in trehalose metabolism	
<i>treZ</i>	2	1	2	1	1	Additional genes involved in trehalose metabolism	
<i>treS</i>	1	1	1	1	0	Additional genes involved in trehalose metabolism	
<i>glsB/yeaQ/ymgE</i>	4	4	5	3	3	GlsB/YeaQ/YmgE family stress response	
<i>osmC</i>	4	3	4	4	1	OsmC family protein/peroxidase activity/response to oxidative stress/hyperosmotic	
<i>msrA</i>	2	2	4	2	2	peptide-methionine (S)-S-oxide reductase MsrA	
<i>msrB</i>	2	2	2	2	3	peptide-methionine (R)-S-oxide reductase MsrB	
<i>acs</i>	1	2	1	1	1	Acetyl-CoA synthetase for acetate metabolism during stress	Ion Transporters and Channels
<i>kdpA</i>	1	1	1	1	1	High-affinity potassium uptake system	
<i>kdpB</i>	1	1	1	1	1	High-affinity potassium uptake system	
<i>kdpC</i>	1	1	1	1	1	High-affinity potassium uptake system	
<i>kdpD</i>	1	1	1	1	1	High-affinity potassium uptake system	
<i>kdpE</i>	1	1	1	1	1	High-affinity potassium uptake system	
<i>kup</i>	2	2	3	1	3	potassium transporter Kup	
<i>nhaA</i>	0	1	0	0	1	Sodium/proton antiporters for ion homeostasis	Stress-Responsive Regulatory Genes
<i>nhaD</i>	0	3	1	0	0	sodium:proton antiporter NhaD	
(Na ⁺ /H ⁺ antiporter)	3	0	3	2	3	Na ⁺ /H ⁺ antiporter	
<i>typA</i>	1	1	1	1	1	response to stress/translational GTPase TypA	
<i>rssB</i>	3	2	2	0	4	Regulator of RpoS/Sigma factor for general stress response	
<i>ompR</i>	6	4	3	0	7	Two-component system regulating osmolarity	
<i>envZ</i>	0	0	0	0	1	Two-component system regulating osmolarity	
<i>cpxA</i>	1	0	1	0	0	Two-component system for envelope stress response	DNA Repair and Protection
<i>dps</i>	0	1	0	1	1	DNA-binding protein protecting DNA during stress	
(Universal stress protein)	11	10	6	5	5	universal stress protein	
<i>recA</i>	1	1	1	1	1	DNA repair and recombination protein	
<i>uvrA</i>	2	1	2	1	2	DNA repair system for UV and oxidative damage	
<i>uvrB</i>	1	1	1	1	1	DNA repair system for UV and oxidative damage	
<i>uvrC</i>	1	1	1	1	1	DNA repair system for UV and oxidative damage	Chaperones and Proteases
<i>dnaK</i>	1	1	1	1	2	Heat shock proteins acting as molecular chaperones	
<i>dnaJ</i>	1	1	1	2	4	Heat shock proteins acting as molecular chaperones	
<i>hrcA</i>	1	1	1	1	1	heat-inducible transcriptional repressor HrcA	
<i>hspQ</i>	1	1	1	1	1	heat shock protein HspQ	
<i>grpE</i>	1	1	1	1	1	Heat shock proteins acting as molecular chaperones	
<i>clpB</i>	1	1	1	1	1	Proteases and chaperones for protein refolding/degradation	
<i>clpX</i>	1	1	1	1	2	Proteases and chaperones for protein refolding/degradation	Antioxidant Defense
<i>clpP</i>	1	1	2	1	1	Proteases and chaperones for protein refolding/degradation	
<i>ibpA</i>	3	4	5	4	4	Small heat shock proteins	
<i>sodA</i>	0	1	0	1	1	Superoxide dismutase for oxidative stress tolerance	
<i>sodB</i>	1	1	1	1	1	Superoxide dismutase for oxidative stress tolerance	
<i>katG</i>	3	1	3	3	1	Catalases for hydrogen peroxide detoxification	
<i>ahpC</i>	0	1	1	1	1	Alkyl hydroperoxide reductase	Cell Envelope Modifications
<i>lpxC</i>	1	1	1	1	1	Genes involved in lipid A biosynthesis for membrane stability.	
<i>lpxD</i>	1	3	1	1	3	Genes involved in lipid A biosynthesis for membrane stability.	

Table S6: Characteristics of *nif*-gene clusters in *Bradyrhizobium* sp. strains BA2, RDT10, RDT46, RDI18 and TZ2

strain	Average Genome % GC	%GC <i>nif</i>-cluster	Cluster type
RDT46	64.8	62.5	SC
BA2	63,08	59,99	SC
RDT10	62,09	60,92	SC
RDI18	61,96	62	NSC
TZ2	61,7	59,1	SC

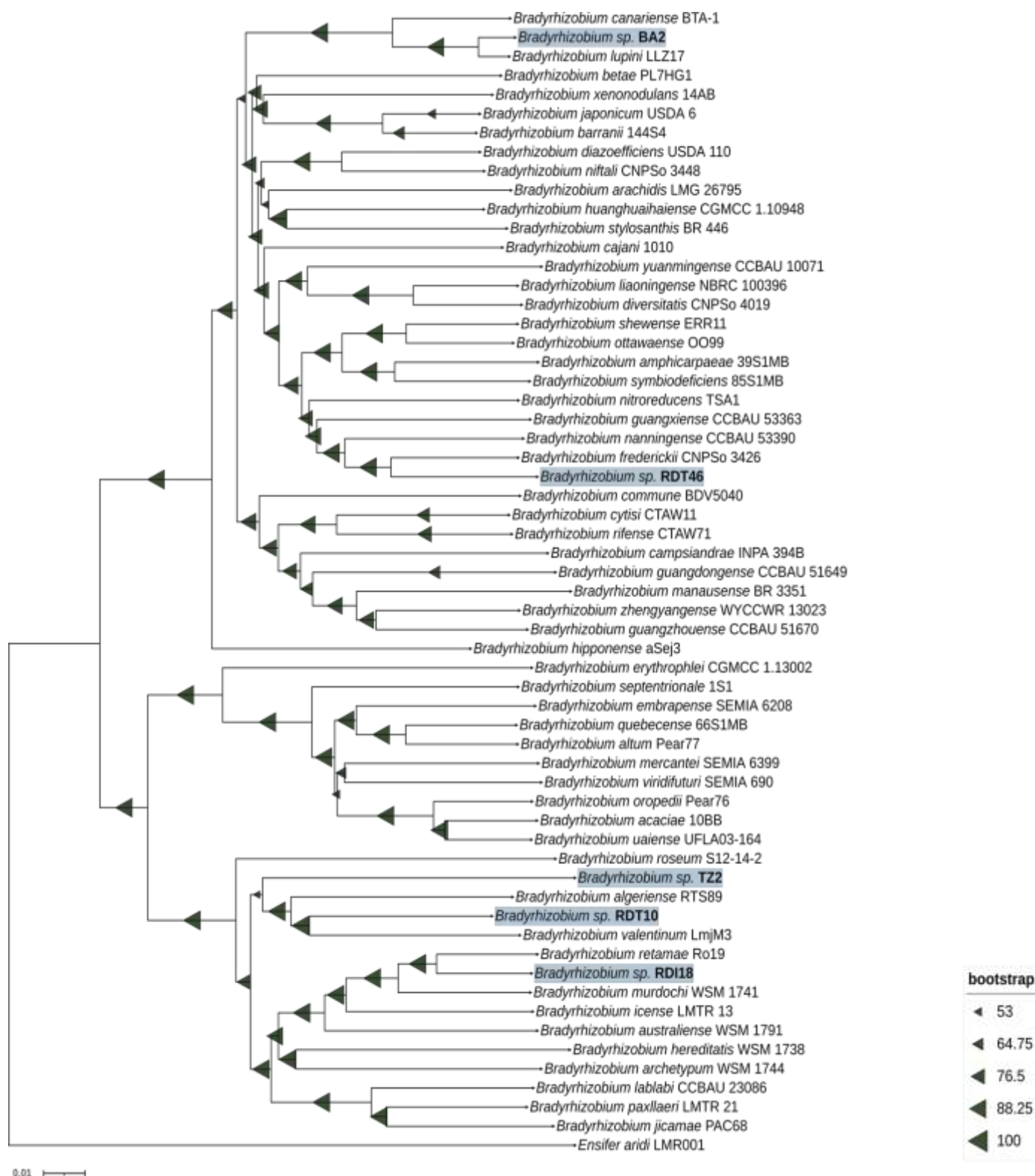


Fig. S1 Likelihood phylogenomic tree based on the whole-genome sequences of *Bradyrhizobium* sp. strain BA2, RDI18, RDT10, RDT46 and TZ2 and their closely related *Bradyrhizobium* type strains.

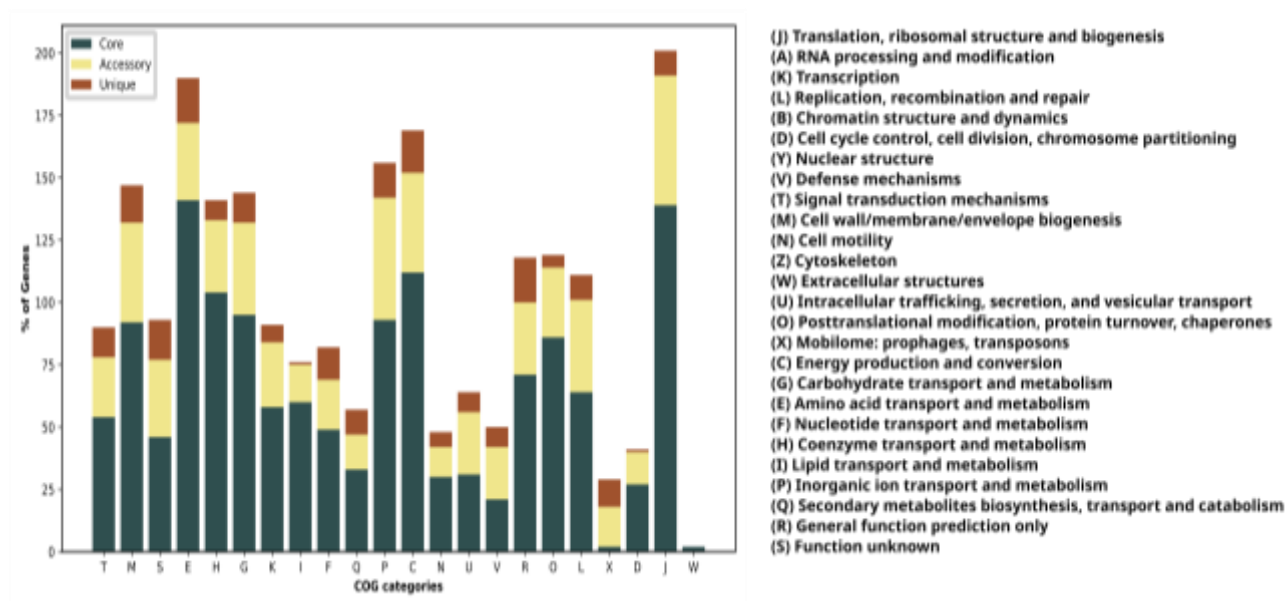


Fig. S2 Bar plot showing the proportion of core, accessory, and unique genes across COG functional categories in five bacterial strains.

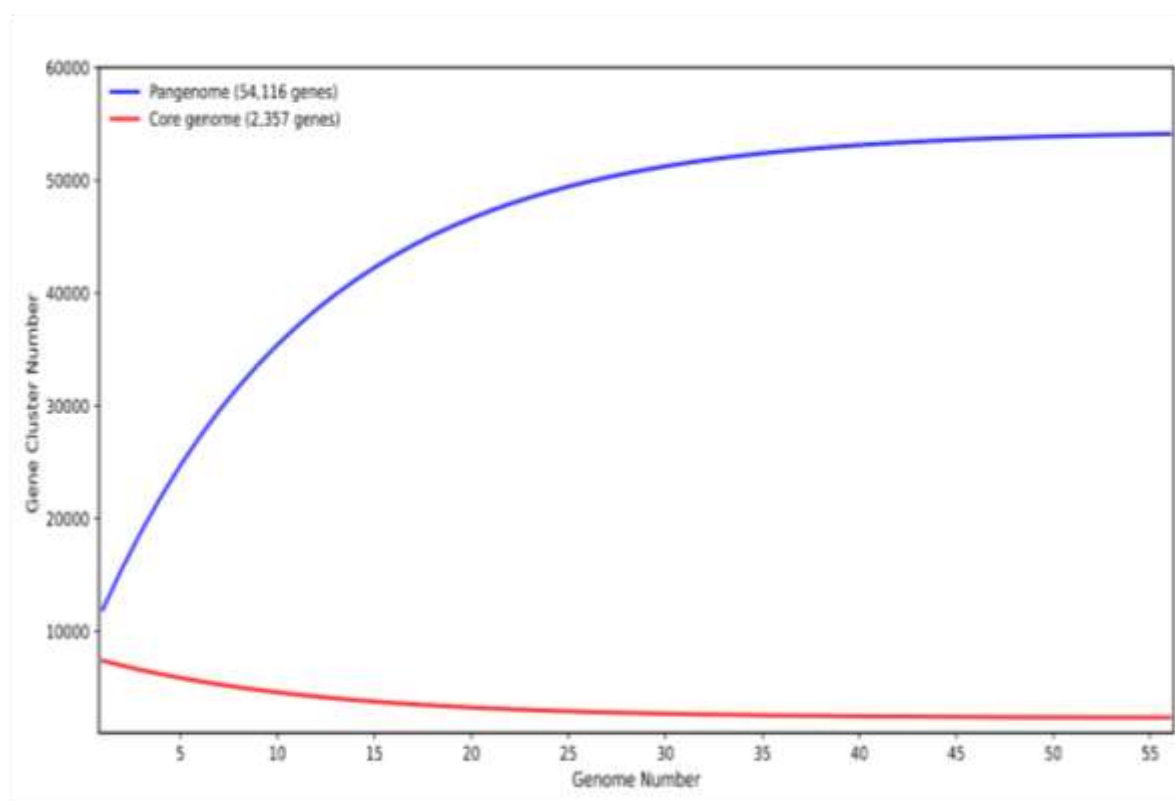


Fig. S3 Core and pan-genome cumulative curves of 56 symbiotic *Bradyrhizobium* species

