A DATABASE FOR TAXONOMIC AND PHYLOGENETIC IDENTIFICATION OF THE GENUS BRADYRHIZOBIUM USING MULTILOCUS SEQUENCE ANALYSIS

1 TUTORIAL

In this tutorial we'll show you how to navigate through some of the available options.

2 ABOUT

On the main page of our database, you will find an overview of the work that led to this site.

Biological nitrogen fixation, with an emphasis on the legume-rhizobia symbiosis, is a key process for agriculture and the environment, allowing the replacement of nitrogen fertilizers, reducing water pollution by nitratE as well as emission of greenhouse gases. Soils contain numerous strains belonging to the bacterial genus Bradyrhizobium, which establish symbioses with a variety of legumes. However, due to the high conservation of Bradyrhizobium 16S rRNA genes-considered as the backbone of the taxonomy of prokaryotes-few species have been delineated. The multilocus sequence analysis (MLSA) methodology, which includes analysis of housekeeping genes, has been shown to be promising and powerful for defining bacterial species, and, in this study, it was applied to Bradyrhizobium species, increasing our understanding of the diversity of nitrogen-fixing bacteria.

Classification of bacteria of agronomic importance is relevant to biodiversity, as well as to biotechnological manipulation to improve agricultural productivity. We propose construction of an on-line database that will provide information and tools using MLSA to improve phylogenetic and taxonomic characterization of Bradyrhizobium, allowing the comparison of genomic sequences with those of type and representative strains of each species.

A database for the taxonomic and phylogenetic identification of the *Bradyrhizobium* genus, using MLSA, will facilitate the use of biological data available through an intuitive web interface. Sequences stored in the on-line database can be compared with multiple sequences of other strains with simplicity and agility through multiple alignment algorithms and computational routines integrated into the database. The proposed database and software tools can be used, free of charge, by researchers worldwide to classify *Bradyrhizobium* strains; the database and software can be applied to replicate the experiments presented in this study as well as to generate new experiments. The next step will be expansion of the database to include other rhizobial species.

2.1 URL, AND CONTACT

Clicking on the About tab will show you the Contact sub-menu, which contains contact data of the main researcher.

- 2.1.1 URL: http://mlsa.cnpso.embrapa.br
- 2.1.2 Important: This website and its applications are best viewed in Firefox browser version 30 or later.
- 2.1.3 **Contact**: Contact for information about the website and database.

Name: Fabrício Martins Lopes E-mail: fabricio@utfpr.edu.br URL: http://www.utfpr.edu.br/cornelioprocopio

3 SEQUENCES DOWNLOAD

Clicking on the Sequences tab will show you the Download sub-menu, which will link you to the Data Table component, with all sequences stored in our database.

The database currently has 286 entries, distributed as follows:

- For all (57) strains stored, we provide four genes (atpD, dnaK, glnII and recA);
- For 30 strains stored, we provide five different genes (atpD, dnaK, glnII, recA and gyrB);
- For 28 strains stored, we provide four different genes (atpD, dnaK, glnII, recA, gyrB and rpoB).
- 3.1.1 Ordering can be changed by clicking on the arrows that appear to the right of each field in the table's first row.

A datal	base for the taxonomic and phylogenetic identification of the genus Bradyri	nizobium using	multilocus sequer	nce analysi	5
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Gene *	Description	Length Sequence	Access Number	Download	NCBI
atpD	Bradyrhizobium inomotense atpD gene for ATP synthase beta subunit, partial cds.	507	AB300994		+
atpD	Bradyrhizobum japonicum partial atpD gene for ATP synthase beta chain.	485	AM168320		+
atpD	Bradyrhizobium canariense bv. genistearum strain BTA-1 ATP synthase beta subunit (atpO) gene, partial cds.	483	AY386739		+
atpD	Bradythizobium liaoningense by glycinearum strain LMG 18230 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY366752		+
atpD	Bradyrhizobium eikanii strain USDA 76 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386758		+
atpD	Bradyrhizobium yuanmingense strain CCBAU 10071 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386760		+
atpD	Bradyrhizobium japonicum strain SEMIA 511 ATP synthase beta subunit (atpD) gene, partial cds.	576	FJ390942		+
atpD	Bradyrhizobium japonicum strain SEMA 512 ATP synthase beta subunit (atpD) gene, partial cds.	576	FJ390943		+

3.1.2 You can also search for specific organisms using keywords in the Search field on the right.

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Gene	Description	Length Sequence	Access Numbe	r Download	NCBI
atpD	Bradyrhizobium betae partial mRNA for ATP synthase F1 (atpD gene), type strain LMG 21967T.	504	FM253129		+
dnaK	Bradyrhizobium betae strain PL7HG1 Hsp70 class chaperone (dnak) gene, partial cds.	598	AY923046		+
ginli	Bradyrhizobium betae ginil gene for glutamine synthetase II, partial cds. strain: LMG 21987.	637	AB353733		+
gyrB	Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T.	669	FM253217		+
recA	Bradyrhizobium betae recA gene for recombinase A, partial cds, strain: LMG 21987.	508	AB353734		+
	Bradyrhizobium betae partial rooB gene for RNA polymerase, beta subunit, type strain LMG 21987T.	1440	FM253260		+

3.1.3 By clicking on the download check box (on the right of each strain) will give file in Fasta format, for one or more sequences.

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Gene	Description	Length Sequence	Access Number	Download	NCBI
atpD	Bradyrhizobium betae partial mRNA for ATP synthase F1 (atpD gene), type strain LMG 21987T.	504	FM253129		-
dnaK	Bradyrhizobium betae strain PL7HG1 Hsp70 class chaperone (dnaK) gene, partial cds.	596	AY923046		+
ginit	Bradyrhizobium betae ginli gene for glutamine synthetase II, partial cds, strain: LMG 21987.	637	AB353733		+
gyr8	Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21967T.	669	FM253217		-
recA	Bradyrhizobium betae recA gene for recombinase A, partial cds, strain: LMG 21987.	508	AB353734		+
				122	

3.1.4 By clicking the download check box button will give file in Fasta Format, for all sequences displayed.

Gene	Description	Length Sequence	Access Number	Download	NCBI
atpD	Bradyrhizobium canariense bv. genistearum strain BTA-1 ATP synthase beta subunit (atpO) gene, partial cds.	483	AY386739	8	+
atpD	Bradyrhizobium liaoningense bv. glycinearum strain LMG 18230 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386752	2	+
atpD	Bradyrhizobium elkanii strain USDA 76 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386758	2	+
atpD	Bradyrhizobium yuanmingense strain CCBAU 10071 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386760		+
atpD	Bradyrhizobium japonicum partial atpD gene for ATP synthase beta chain.	485	AM168320		+
atpD	Bradyrhizobium inomotense atpD gene for ATP synthase beta subunit, partial cds.	507	AB300994	2	+
atpD	Bradyrhizobium pachyrhizi strain PAC48 AtpD (atpD) gene, partial cds.	512	FJ428208	2	+
atpD	Bradyrhizobium jicamae strain PAC68 AlpD (atpD) gene, partial cds.	512	FJ428211		+
atpD	Bradythizobium betae partial mRNA for ATP synthase F1 (atpD gene), type strain LMG 21987T.	504	FM253129	×	+
atpD	Bradyrhizobium japonicum SEMIA 5079 ATP synthase beta subunit (atpD) gene, partial cds.	576	F./390956	8	+
atpD	Bradyrhizobium diazoefficiens SEMA 5050 ATP synthase beta subunit (atpD) gene, partial cds.	576	FJ390957	8	+

3.1.5 The Next Step is selected click download button to download the desired sequences into a single file.

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3.1.6 Clicking on the NCBI button (on the right of each strain) will give you access to the desired sequence in the NCBI.

A datat	base for the taxonomic and phylogenetic identification of the genus Brady	rhizobium usin	ng mu	Itilocus seque	nce analysi	s
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atpD	Bradyrhizobium betae partial mRNA for ATP synthase F1 (atpD gene), type strain LMG 21987T	504		FM253129	8	+
dnaK	Bradyrhizobium betae strain PL7HG1 Hsp70 class chaperone (dnaK) gene, partial cds.	598		AY923046		+
ginit	Bradyrhizobium betae ginil gene for glutamine synthetase II, partial cds, strain: LMG 21987.	637		AB353733	1	+
gyrB	Bradymizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T.	669		FM253217		-
recA	Bradyrhizobium betae recA gene for recombinase A, partial cds, strain. LMG 21987.	508		AB353734		+
rpoB	Bradyrhizobium betae partial rpoB gene for RNA polymerase, beta subunit, type strain LMG 21987T	1440		FM253260		+
Showing 1 Download	to 6 of 6 entries (filtered from 286 total entries) selected					

3.1.7 On the upper corner of this page you find the possible format options for the GenBank.

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	type strain LMG 21987T.	Shaw several complement	
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	Bradyrhizobiaceae; Bradyrhizobium.	Due BLAST	
REFERENCE	1	Rui Gosti	
AUTHORS	Rivas,R., Martens,M., de Lajudie,P. and Willems,A.	PICK Primers	
TITLE	Multilocus sequence analysis of the genus Bradyrhizobium	Highlight Sequence Features	
JOURNAL	Syst. Appl. Microbiol. 32 (2), 101-110 (2009)	Find in this Sequence	
PUBLED	2 (bases 1 to 669)		
AUTHORS	Nilema A.M.C.G.		
TITLE	Direct Submission	LinkOut to external resources	
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		DNA ovrase B subunit type str	rain Music

- 3.1.8 There are two ways to transfer the data to your device:
- 3.1.8.1 By selecting and copying the sequence data right clicking the mouse;

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3.1.8.2 Or by the Send dialog box in the right upper corner of the screen and selecting "complete record" and "file".



4 ANALYZE

In this part of tutorial, we will show you the process of multilocus sequence analysis.

4.1.1 Clicking the context menu "analysis" will show you the option "align with strain MLSA".



4.1.2 In the first dialogue box on your left, clicking on the arrow will give you the choice between Clusta Omega or Muscle.

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- Select gene	¥	
Select the fifth gene:		Paste your sequence for fifth gene
- Select gene	×	
		Paste your sequence for sixth gene
Select the sixth gene:		

4.1.3 The next six dialogue boxes on left allow you to select each of the one to six genes to be used for analysis.

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Select the fourth gene	Paste your sequence for fourth gene
- Select gene -	-
Select the fifth gene:	Paste your sequence for fifth gene
Select gene	
Select the sixth gene	Paste your sequence for sorth gene
Select gene	

4.1.4 In this example we must select 3 different genes and paste the sequences for each of them in the "field " paste your sequence for your first (second / third) gene.

About Sequences		nalyze Help
Multiple Sequence a Select the algorithm:	Align	iment
Clustal Omega		
Select the first gene:		Paste your sequence for first gene (min 455 bp):
atpD	3	Cetta-Astrocavatrococctoraterteseconavita-Aconecocccessocccossocccostradors certa-costertesecontresocs-Ansarctrococcacanagesccasgaccategetartetestrocacamica tettecocttea-ce
Select the second gene		Paste your sequence for second gene (min 369 bp)
dnaK	2	
Select the third gene		Paste your sequence for third gene (min 538 bp)
ginit	3	CetTDBAMAAAACATCTCAACTTCTBCCTCGCGGCCGGGATCAACCATCAAAGCCATCAACGCATAAATTGCATC AAAGGOCCATGGGATCCAACTTCTGCGCGCGGAGGCAGCAACGACGACGACGACAACTGCGATGGCGC GCTACCTCAATGCTGCGCCCTGACCGACGACGACGCCGCCGCGCGA CACCGAC
Select the fourth gene:		Paste your sequence for fourth gene
— Select gene —		
Select the fifth gene:		Paste your sequence for fifth gane
- Select gene	3	
Select the sixth gene		Paste your sequence for sixth gene
Select gene	÷	

4.1.5 When selecting a particular gene, information on the smallest size of the sequence stored: min xxx bp (example: min 369 bp) which means that the smallest sequence for gene dnaK in the databank has 369 base pairs.

Multiple Sequence Select the algorithm:	ce Aligr	ament	
Clustal Omega	2		
Select the first gene:		Paste your sequence for first gen (min 455 bp)	
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Select the second gene:		Paste your sequence for second gery (min 369 bp).	
dnaK	×	ACOCCITOCATCGAGGATOCCOCHACCHACCHACGAAGCGCTGAAGGGCGACGATGGCCAAGGCGACC AAGGCCAAGACCAGACGCTGGGCCAAGGACGTCGATGAAGCTCGGCGAGGCCATGTACACGAAGAGGCCG AGGCCGACGCCAAGAAGGATGCGGCCAAGGACGACGTC	•
Select the third gene:		Paste your sequence for third ger (min 538 bp)	
gini	3	CGTGAMAAAACATTCXACCTCTCCCCTCOCCCCCCCCCCCCCCCCC	• •

4.1.6 After filling in your sequences, you can click the send button and wait for results.

About 5	sequences	Analyze	Help	
Multiple Se Select the algori	quence Al	ignment		
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atpD		CGTCA CTCAC TCTTO	MATEGANASTECORDETGETTEGECANTGANCEACCCCCCGGCCCCCCCCCCCCCCCCCCCCCCCCCC	Û
Select the second	nd gene	Paste y	rour sequence for second gene (min 369 bp)	
dnaK		ACGCO AAGGO AGGO	CGTCCCATCGAGGATGCCGTCAACGACCTCAAGGAACGACTGAAGGGCGACGATGCCGAGGCGATC CCAACACCCAGACGCTGCCCCAGGCTTCGATGAAGCTCGGCGAGGCCATGTACACGCAGGAGGCCG CGACGCCAAGAAGGATGCGGCCAAGGACGACGTC	Û
Select the third	gene:	Paste y	rour sequence for third gene (min 538 bp):	
gini		CGTGC AAGO GCTAC CACCO	BARGABENTCTEGACTETECETESEGGECESGEATCAACCATGANGEDTCAACDOGAAGTCGCC GCCATGGGGAATCCAGAUTTCGGCAAGGGCTCCAAGAGGCCCCCGGACGAAATGTGGATGGCCC CTGATGCTGCGCCTGACCGAGAAGTACGGCATCGACATCGACATCGACATCGACGGCCGGC	Ĵ
Select the fourth	gene	Paste y	rour sequence for fourth gene	
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Select the fifth g	iene:	Paste y	rour sequence for fifth gene	
- Select gene	-	~		
Select the sixth	gene	Paste y	rour sequence for soth gene	
- Select gene	-/	2		

4.1.7 A screen informs the procedure being performed.



- 4.1.8 The results will be shown in the following order (Alignment, Identity Matrix and Parameters for the Generation of the Phylogenetic Tree), and include download option to alignments, the identity matrix and the newick tree.
- 4.1.8.1 ALIGNMENT

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4.1.8.2 IDENTITY MATRIX

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SEMIA 6319	93.31	93.53	93.66	89.34	97.05	94.27	93.54	65.06	91.33	93.31	\$4.42	\$4.94	95.02	82.21
SEMIA 6374	94.09	94.70	96.13	89.08	94.95	95.13	93.46	85.69	90.40	94.09	95.29	96.44	98.52	93.3
SEMIA 6434	94.71	93.85	93.23	89.90	93.22	94.35	96.58	68.95	91.58	94.71	94.51	94.71	94.79	94.88
SEMIA 6440	87.76	87.62	88.62	97.51	89.42	88.82	88.88	97.98	89.99	87.76	88.82	88.63	88.79	89.03
SEMIA 0144	94.27	94.15	95.36	89.06	95.18	94.00	93.07	85.45	89.55	94.27	94.19	95.59	95.67	92.29
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4.1.8.3 PARAMETERS FOR GENERATION OF THE PHYLOGENETIC TREE.

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				Opening Phylogen	tic-Tree.txt			
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			What should Firefo	w do with this file?				
			O Open with	Notepad (default)				
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4.1.9 After downloading, you can use programs for data processing.

4.1.10 Next step is an example alignment and phylogenetic tree analysis using MEGA software version 6.

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DNA Sequences Translated Protein Seque	inces													
Species/Abbrv	Grou	p Name		••••	• •	•••	•		• •	• •	•		• •	• •
1. USER_SEQUENCE				Seco	CCA	t c c	AC	1	110	cec	848	C A C I	CC .	ACC.
 B.canariense_LMG_22265 				COCO	CCA	t c c	Acc	X 2 0	A A 8	c e c(848	C A C J	CC .	A C C
 B.liaoningense_LM0_18230 				cec	CCA	t c c	Acc	100	X 0 0	CAC	848	C A C J	CC .	ACC
 B.elkanii_USDA_76 				COCO	CCA	t c c	ABC			CAC		C A C I	CC .	ACC.
 B.yuanmingense_LMG_21027 				COCO	CCA	t c c	Acc	X 2 2		c e c(C ACI	C A C I	CC .	ACC.
B.japonicum_USDA_6				cec	CCA	t c c	AC	100	X 0 0	coc	a C	C AC	CC .	ACC
B.iriomotense_EK05				COC	C I A	t c c	Acc			c e c(B ACI		CC .	ACC.
 B.pachyrhizi_PAC_48 				COCO	CCA	t c c	AC	1		c e c(B ACI	C AC	CC .	.
9. B.jicamae_PAC_68				COC	CCA	t c c	Acc			c e c(B ACI		CC .	ACC.
10. B.betae_LNG_21987				cecc	CCA	t c c	A 🛛 🕻			C O C(C AC	CC.	ACC.
11. SEMIA_5079				cece	CCA	E C C	Acc	1		c e c(A C	C A C	CC .	ACC.
12. SEMIA_5080				cec	CCA	t c c	Acc	100		c e c(a a c	C A C J	CC .	Acc
13. SEMIA_6059				COC	CCA	t c c	Acc			c e ci	C A C	C A C I	CC .	ACC
14. B.cytisi_CTAN11				cec	CCA	E C C	AC	1		coc	848	C AC	CC .	ACC
15. B.rifense_CTAN71				6666	C D A		ACC	100	110	coci	. 00C			ACC.
¢														>

4.1.11 Use option DATA > Export Alignment > MEGA Format

Data Edit Search Alignment	Search Alignment Web Sequencer Displa	y Help	
Create New Reconstruction Reconstruction Reconstruction Phylogenetic Analyte Seve Session Ctrls> Seve Seve Seve Seve Seve Seve Seve Seve	New W W W L In Geogenese In Geogenese Sission Ctrl-3 Alignment KASTA Format Reparces PAUP Format Reparces Complement Bandt Ce Table Complement anement Supjoreer		

4.1.12 Save as alignment.meg



4.1.13 Input title of the data: "betae_test"



4.1.14 Protein-coding nucleotide sequence data? "Yes"



4.1.15 Now Open Phylogeny Tab in Software Mega.

4.1.16 Choose a Data File to Analyze: "alignment.meg"

۱		Choose a Data File to Anal	lyze		×
🕣 - † 🎴	This PC > Local Disk (C:) > MLSA		~ C	Search MLSA	۵
Organize • New	folder			III 🔹 📶	
🚖 Favorites	Name	Date modified	Type Size		
Desktop	Alignment.meg	10/26/2014 2:54 PM	MEG File	83 KB	
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 4.1.17 In Options Summary, input 3 parameters different of default analysis preferences: "Test Phylogeny: Bootstrap method", "No. of Bootstrap Replications: 1000" and "Model/Method: Tamura-Nei model". Click in compute.

options Summary	
Option	Selection
Analysis	Phylogeny Reconstruction
Scope	All Selected Taxa
Statistical Method	Neighbor-joining
Phylogeny Test	
Test of Phylogeny	Bootstrap method
No. of Bootstrap Replications	1000
Substitution Model	
Substitutions Type	Nucleotide
Genetic Code Table	Not Applicable
Model/Method	Tamura-Nei model
Fixed Transition/Transversion Ratio	Not Applicable
Substitutions to Include	d: Transitions + Transversions
Rates and Patterns	
Rates among Sites	Uniform rates
Gamma Parameter	Not Applicable
Pattern among Lineages	Same (Homogeneous)
Data Subset to Use	
Gaps/Missing Data Treatment	Complete deletion
Site Coverage Cutoff (%)	Not Applicable
Select Codon Positions	✓ 1st ✓ 2nd ✓ 3rd ✓ Noncoding Sites

4.1.18 A screen informs the procedure being performed

	80%	
Details 🔻	X gtop	
Status / Options		
Run Status		
Start time	10/26/2014 3:00:13 PM	
Status	Conducting Bootstrap Test	
Bootstrap Rep #	603/1000	
Analysis Options		
Analysis Options Analysis Analysis	Phylogeny Reconstruction	
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Analysis Options Analysis Scope	Phylogeny Reconstruction All Salected Taxa Method Neighbor-Joining te logeny Bootstrap method strap Replications 1000	

4.1.19 Result of analysis:



4.1.20 To make an analysis of Identity-Matrix.csv file, can be used a software Open Calc of Apache OpenOffice software available in: <u>http://www.openoffice.org/</u>



4.1.21 To the phylogenetic analysis of the file "Phylogenetic-Tree.txt" an option can be found in the services available on the website: <u>http://www.trex.uqam.ca/</u>



5 COMMENTS AND SUGGESTIONS

5.1 WE WOULD BE VERY HAPPY WITH YOUR COMMENTS OR SUGGESTIONS.

PLEASE CONTACT US.