

# List of Appendices

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# Appendix-A

## Thesis related publications and genome sequencing project

Rai, R and Sen, A(2015) Biochemical Characterization of French Bean Associated *Rhizobium* found in North Bengal and Sikkim. *Journal of Academia and Industrial Research*, 4(1):10-18.

Rai, R. Bantawa, P. Sur, S. and Sen, A(2014) Trends in biochemical and molecular characterization of rhizobia and their Nitrogen Fixation mechanism: A review. *Biology of useful plants and microbes*, 61-119.

**Whole Genome Bioproject** Accession: PRJNA296467

Biosample Accession: SAMN04099707

**Genome Id: 2684623037**

## Other publications

Saha, M. Rai, R. Kar, P. Sen, A and Sarker, D (2015) Ethnobotany, traditional knowledge and socioeconomic importance of native drink among the Oraon tribe of Malda district in India. *Journal of Intercultural Ethnopharmacology*, 4(1): 34-39.

Thakur, S. Sur, S. Bose, D. Goyal, AK. Mishra, T. Rai, R. Bhattacharya, M. Bothra, AK and Sen, A (2009) Molecular modelling of a pathogenesis related protein from *Solanum tuberosum*. *NBU Journal of Plant Sciences*, 3:15-20.

# Appendix B

## List of abbreviations

%	Percent
/	Per
°C	Degree celsius
$\mu\text{mol l}^{-1}$	Micro mole per liter
3'→5'	3 prime to 5 prime
5'→3'	5 prime to 3 prime
v/v	Volume by volume
w/v	Weight by volume
$\alpha$	Alpha
$\mu\text{g}$	Microgram
$\mu\text{l}$	Microlitre
$\mu\text{mol}$	Micromole
gm	Gram(s)
gm/l	Gram(s) per litre
hr	Hour(s)
ha	Hectare(s)
mg	Milligram
min	Minute(s)
ml	Millilitre(s)
mM	Millimolar
mm	Millimeter
$\mu\text{M}$	Micromolar
cm	Centimeter
kb	Kilo base pair
M	Molar
sec	Second(s)
rpm	Revolution per minute
ddH <sub>2</sub> O	Sterile distilled water

pH	Negative logarithm of Hydrogen concentration (Measurement of acidity and basicity)
YEMA	Yeast extract mannitol agar
O.D.	Optical Density
CLSI	Clinical Laboratory Standards Institute
NCCLS	National Committee for Clinical Laboratory Standards
CTAB	Cetyl Trimethyl Ammonium Bromide
CD	Critical Difference
DAS	Days after sowing
IS	Intermediately Susceptible
PGPR	Plant Growth Promoting Rhizobia
IAA	Indole-3-acetic acid
CAS	Chrome-azurol S
HCN	Hydrogen cyanide
DNA	Deoxyribose Nucleic Acid
RNA	Ribose Nucleic Acid
PCR	Polymerase chain reaction
RAPD	Random Amplified Polymorphic DNA
REP-PCR	Repetitive Extragenic Palindromic Polymerase chain reaction
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus Sequences Polymerase chain reaction
RFLP	Restriction Fragment Length Polymorphism
16SrRNA	16S ribosomal ribose nucleic acid
RAxML	(Randomized Accelerated Maximum Likelihood, version
EMBL	European Molecular Biology Laboratory
NCBI	National Centre for Biotechnology Information
IMG	Integrated Microbial Genome
KEGG	Kyoto Encyclopedia of Genes and Genomes
CAI	Codon Adaptation Index
RSCU	Relative Synonymous Codon Usage
COGs	Cluster of Orthologs groups
BLAST	Basic Local Alignment Search Tool
RAST	Rapid Annotations using Subsystems Technology

# Appendix-C

## Composition of chemicals used for biochemical tests.

Composition of Yeast Extract Mannitol (YEM) agar medium (Hi Media, Cat# 716)

Sl. No.	Chemical name	Quantity
1	Mannitol	10 g
2	Dipotassium hydrogen phosphate	0.5 g
3	Magnesium sulphate	0.2 g
4	Sodium chloride	0.1 g
5	Yeast extract	1.0 g
6	Congo red (1% solution)	2.5 ml
7	Agar	20.0 g
8	Distilled water	1000 ml

### Modified nitrogen free Jensen's medium

CaHPO <sub>4</sub>	1.0gm	Hi-Media, Cat#RM1109-500G
K <sub>2</sub> HPO <sub>4</sub>	0.2gm	Hi-Media, Cat#RM3943-500G
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2gm	Hi-Media, Cat#RM684-500G
NaCl	0.2gm	Hi-Media, Cat#RM853-500G
FeCl <sub>3</sub>	0.1g	Hi-Media, Cat#RM6353-500G
Trace (elements 1 ml):		
• B	0.05%	Hi-Media, Cat#RM10441-25G
• Mn	0.05%	Hi-Media, Cat#RM3051-250G
• Zn	0.005%	Hi-Media, Cat#RM1435-500G
• Mo	0.005%	Hi-Media, Cat#RM2558-25G
• Cu	0.002%	Hi-Media, Cat#RM720-500G
• Distilled water	1 L	

### Lactose agar medium

Lactose (C <sub>12</sub> H <sub>22</sub> .H <sub>2</sub> O)	10.0gm	Hi-Media, Cat#RM565-500G
KH <sub>2</sub> PO <sub>4</sub>	5.0gm	Hi-Media, Cat#RM3943-500G
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2gm	Hi-Media, Cat#RM3943-500G
NaCl	0.1gm	Hi-Media, Cat#RM853-500G
Yeast extracts	1.0gm	Hi-Media, Cat#M1824-500G
Agar	15.0gm	Hi-Media, Cat#RM026P-500G
Distilled water	1L	

<b>Urea agar medium</b>		
Peptone	1.0gm	Hi-Media, Cat#PTC0407-500G
Sodium chloride	5.0gm	Hi-Media, Cat#RM853-500G
Potassium monohydrogen phosphate	2.0gm	Hi-Media, Cat#RM3943-500G
Glucose	1.0gm	Hi-Media, Cat#RM077-500g
Urea 20% aqueous solution	100ml	Hi-Media, Cat#RM633-500G
Phenol red	0.2% solution	Hi-Media, Cat#RM085-25G
Agar	20.0gm	Hi-Media, Cat#RM026P-500G
ddH <sub>2</sub> O	1L	

<b>Czapek-mineral salt agar medium</b>		
NaNO <sub>3</sub>	2.0gm	Hi-Media, Cat#RM416-500G
K <sub>2</sub> HPO <sub>4</sub>	1.0gm	Hi-Media, Cat#RM3943-500G
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5gm	Hi-Media, Cat#RM3943-500G
KCl	0.5gm	Hi-Media, Cat#RM698-500G
Carboxymethyl cellulose CMC	5.0gm	Hi-Media, Cat#RM9354-500G
Peptone	2.0gm	Hi-Media, Cat#PTC0407-500G
Agar	20.0gm	Hi-Media, Cat#RM026P-500G
Distilled water pH adjusted to 6.5	1L	

<b>Sl. No.</b>	<b>Chemical name</b>	<b>Quantity</b>
1	Peptone	5.0 g/L
2	Beef extract	3.0 g/L
3	Gelatin	12.0 g/L

<b>MOPS-salt MS medium</b>		
3-N -Morpholino propanesulfonic acid buffer	8.37gm	Hi-Media, Cat#RM660-25G
KOH	1.2gm	Hi-Media, Cat#RM1015-500G
NH <sub>4</sub> Cl	1.07gm	Hi-Media, Cat#RM717-500G
MgSO <sub>4</sub>	0.48gm	Hi-Media, Cat#RM1281-500G
K <sub>2</sub> HPO <sub>4</sub>	0.21gm	Hi-Media, Cat#RM3943-500G
KH <sub>2</sub> PO <sub>4</sub>	0.05gm	Hi-Media, Cat#RM3943-500G
NaCl	5.0mg	Hi-Media, Cat#RM853-500G
CaCO <sub>3</sub>	20.0mg	Hi-Media, Cat#RM397-500G
H <sub>3</sub> BO <sub>3</sub>	1.0mg	Hi-Media, Cat#RM1224-500G
ZnSO <sub>4</sub> .7H <sub>2</sub> O	1.0mg	Hi-Media, Cat#RM695-500G
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.5mg	Hi-Media, Cat#RM677-500G
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.5mg	Hi-Media, Cat#RM686-500G
NaMoO <sub>4</sub> .2H <sub>2</sub> O	1.0mg	Hi-Media, Cat#RM1721-500G
Ethylenediaminetetraacetic acid EDTA	10.0mg	Hi-Media, Cat#RM1279-500G
Na-Fe-EDTA	2.0mg	Hi-Media, Cat#RM1197-500G
Distilled water	1L	

<b>GPA medium</b>		
Glucose	40.0gm	Hi-Media, Cat#RM077-500G
Peptone	5.0gm	Hi-Media, Cat#PTC0407-500G
Agar	15.0gm	Hi-Media, Cat#RM026P-500G
Distilledwater	1L	

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<b>Hofers Alkaline Medium</b>	
Ingredients	Gms / Litre
Mannitol	10.00
Dipotassium phosphate	0.500
Magnesium sulphate	0.200
Sodium chloride	0.100
Yeast extract	1.000
Thymol blue	0.016
Agar	15.000
Final pH ( at 25°C)	11.0±0.2

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## **Buffers and chemicals used for DNA fingerprinting studies**

### **CTAB/NaCl**

CTAB (Sigma, Cat# H6269)=10gm

NaCl (Merck India, Cat#60640405001730)= 4.1gm

Final volume=100ml

In 80ml double distilled water, 4.1gm of NaCl was dissolved. Slowly 10gm of CTAB was added with continuous heating and stirring until the solution becomes clear. The final volume of the solution was adjusted to 100ml by adding ddH<sub>2</sub>O. The solution was autoclaved at 121°C and 15 psi for 20 mins and stored at room temperature for further use.

### **1X TE**

Tris- Cl (pH 8.0) (i.e. 10Mm) =0.6055gm

EDTA (pH 8.0) (i.e. 1mM) =0.186 gm

Final volume=1000ml

Both the reagents were dissolved separately and finally mixed together and the final volume was made up to 1000ml with double distilled water prior to autoclaving. The buffer was autoclaved at 121°C and 15 psi for 20 mins and stored at room temperature for further use.

### **5X TBE (Tris-borate-EDTA) buffer**

Trizma base (Sigma, Cat# T1503) = 27 gm

Boric acid (Sigma, Cat# 15663)= 13.75 gm

0.5M EDTA (pH 8.0)=1.86 gm

Final volume=1000ml

All the reagents were dissolved separately and finally mixed together and the final volume was made up to 1000ml with double distilled water prior to autoclaving. The buffer was autoclaved at 121°C and 15 psi for 20 mins and stored at room temperature for further use.

### **Sodium acetate(3M)**

Sodium acetate (Sigma, Cat#S-9513)=12.3045gm

Final volume=50ml

In 50ml of ddH<sub>2</sub>O, 12.3045gm of sodium acetate was dissolved and autoclaved at 121°C, 15 psi for 20 mins and stored at room temperature for further use.

### **SDS(10%)**

SDS (Sigma, USA. Cat #L4390)=5gm

Final volume=50ml

In 20ml of ddH<sub>2</sub>O, 5gm of SDS was added and heated to dissolve. The final volume was made upto 50ml and autoclaved for future use.

**Sodium chloride (NaCl) (5M)**

NaCl=14.61gm

Final volume=50ml

**6X gel loading buffer:**

TYPE 3:

0.25% Bromophenol blue (Sigma, Cat# B0126)

0.25% Xylene cyanol FF (Sigma, Cat# X4126)

30% Glycerol (Merck India, Cat#61756005001730) in water

Store at 4°C.

**RNase A:**

The RNase A enzyme (Sigma, Cat# R4875) was dissolved at a concentration of 10mg/ml in 0.01M sodium acetate (Sigma, Cat# S9513) (pH 5.2). The solution was heated at 100°C for 15 minutes in a water bath and allowed to cool slowly to room temperature. The pH was adjusted by adding 1/10 volume of 1M Tris-Cl (pH 7.4) and stored at -20°C for further use.

**Note:** Both 0.01M sodium acetate and 1M Tris-Cl were prepared and autoclaved at 121°C and 15 psi for 20 mins prior to use.

The other chemical used for the molecular work are:

Ready Mix <sup>TM</sup> Taq PCR Reaction (Sigma, Cat# P4600)

Proteinase K (Sigma, USA. Cat #P2308)

Chloroform (Merck India, Cat #822265):

Isoamylalcohol (Merck India Cat# 8.18969.1000)

Phenol (Sigma, Cat #P4557)

Isopropanol (Merck India, Cat#17813)

Absolute ethyl alcohol (BDH, Cat#10107)

Agarose (Sigma, Cat#A9539)

Ethidium bromide (10mg/ml) (Hi Media, Cat# RM813)

Lambda DNA/ EcoRI/ HindIII double digest (Promega, Cat# PR-G1731)

100 bp ladder (Sigma, Cat#1473)

Go Taq Green Master Mix (Promega, Cat# M7122)-

Alu I (Promega, Cat # R6281), EcoRI (Promega, Cat# R6011), HaeIII (Promega, Cat# R6171)

Hinf I (Promega, Cat# R6201), Hpa II (Promega, Cat# R6311), MboI (Promega, Cat# R6711)

Taq I (Promega, Cat# R 6151), PstI (Promega, Cat# R6111).

## **Chemicals used for various PGPR tests of *Rhizobium***

Ferric chloride (Himedia, Cat# RM1379)  
Hydrochloric acid (Merck India, Cat# 61762505001730)  
Hydrogen peroxide (Merck India, Cat# 61765305001730)  
Potassium dihydrogen phosphate (Merck India, Cat# 60487305001730)  
Dipotassium hydrogen phosphate (Merck India, Cat#61788005001730)  
Potassium hydroxide (Merck India, Cat#60503305001730)  
Sodium Carbonate (Merck India, Cat# 61778705001730)  
Sodium hydroxide (Merck India, Cat# 6184305001730)  
Sodium Nitrite (Himedia, Cat# RM417)  
Sulphanilamide (Himedia, Cat# RM1558)  
Pikovskaya's agar medium (Cat# M520, Hi-Media, Mumbai)  
  
Chrome azurol S(CAS) agar medium (Hi-Media, Cat # RM 336).  
  
HDTMA (HiMedia, Cat# RM4867)  
  
PIPES (HiMedia, Cat # RM659)  
  
casamino acid (Bacto, Cat#223120)

# Appendix-D

## Software used in the present study

<b>Name</b>	<b>Executable</b>	<b>Description</b>
CodonW	Windows	Program for codon and amino acid usage
XLSTAT	Windows	Statistical and data analysis software package
CMG-biotools	Linux	Stand alone OS for comparative microbial genomics
MEGA	Windows	Tool for sequence alignment and phylogeny
ClustalW	Windows	Multiple sequence alignment program
Bioedit	Windows	Biological sequence alignment editor
SPSS	Windows	Software package used for statistical analysis
FigTree	Windows	Graphical viewer of phylogenetic trees
BLAST	Windows/linux	Algorithm for local similarity between sequences
R-Package	Windows/linux	Functional language for statistical analysis
KyPlot	Windows	Software for plotting graphs

## Web server used in present study

<b>Name</b>	<b>Web Address</b>	<b>Description</b>
JGI-IMG	<a href="http://www.img.jgi.doe.gov">www.img.jgi.doe.gov</a>	Integrated Microbial Genomes system
NCBI	<a href="http://www.ncbi.nlm.nih.gov/">www.ncbi.nlm.nih.gov/</a>	For molecular biology information
CAI Calculator2	<a href="http://userpages.umbc.edu/~wug1/codon/cai/cais.php">http://userpages.umbc.edu/~wug1/codon/cai/cais.php</a>	Calculation of codon adaptation index
RAST	<a href="http://rast.nmpdr.org">rast.nmpdr.org</a>	For the annotation of whole genome sequence

# Appendix-E

List of Supplementary Material  
(provided in the compact disc attached with the thesis)

## Supplementary Tables

**Table ST1**                    IMG annotation of the whole genome sequenced *Rhizobium* strain RSm-3

## Supplementary Figures

**Figure SF 1**                    Fig 4.27. Dendrogram generated by RAxML representing the relationship of the eleven *Rhizobium* strains with the standard GenBank partial sequences of rhizobial 16SrDNA

**Table ST1: IMG annotation of the whole genome sequenced *Rhizobium* strain RSm-3.**

<b>Strain details</b>	<b><i>Rhizobium</i> sp. RSm-3</b>
IMG Product Assignment	Yes
High Quality	Yes
Cultured	Yes
GOLD Sequencing Strategy	Whole Genome Sequencing
Sequencing Center	University of New Hampshire
Assembly Method	Trimmomatic 0.32, Spades 3.5.0, Allpaths 52415
Biosample Accession	SAMN04099707
Strain	RSm-3
Isolation	root nodules of the French bean ( <i>Phaseolus vulgaris</i> )
Study Name	<i>Rhizobium</i> sp. RSm-3 Genome sequencing
Project / Study Name	<i>Rhizobium</i> sp. RSm-3
Genome Name / Sample Name	<i>Rhizobium</i> sp. RSm-3
Species	<i>Rhizobium</i> sp. RSm3
Genus	<i>Rhizobium</i>
Order	Rhizobiales
Family	Rhizobiaceae
Phylum	Proteobacteria
Bioproject Accession	PRJNA296467
Submission Type	Primary
Host Name	<i>Phaseolus vulgaris</i>
Is Public	No
Gene Model QC	No
Ecosystem Category	Microbial
User Access	LouisTisa, celinepesceSSOAPI, erikswanson91, maher.gtari, rediettamire, senarnab_nbu
Contact Email	louis.tisa@unh.edu
Alt2. Contact Emails	louis.tisa@unh.edu

Alt. Contact Email	louis.tisa@unh.edu
Contact Name	Louis S. Tisa
Alt2. Contact Names	Louis S. Tisa
Alt. Contact Name	Louis S. Tisa
GOLD Sequencing Quality	Level 2: High-Quality Draft
Culture Type	Isolate
Ecosystem	Host-associated
GOLD Study ID	Gs0121357
GOLD Project ID	Gp0153659
GOLD Analysis Project Type	Genome Analysis
GOLD Analysis Project ID	Ga0133937
Status	Draft
Geographic Location	Darjeeling, Himalaya, Sonada, India
Ecosystem Type	Bacteria
Domain	Bacteria
Class	Alphaproteobacteria
Sequencing Method	454; Illumina HiSeq
taxon_oid	2684623037
IMG Genome ID	2684623037
Genome Size assembled	6912093
Coding Base Count	6042552
Coding Base Count NP	5978148
GC Count assembled	4242392
NCBI Taxon ID	1720346
IMG Submission ID	91587
Distance Matrix Calc. Date	42566
Add Date	42563
Gene Count assembled	6564

CDS Count assembled	6511
Chromosomal Cassette Gene Count	6407
Pfam Count assembled	5466
w/ Func Pred Count assembled	5371
Not MetaCyc Count assembled	5135
COG Count assembled	4802
Not KEGG Count assembled	4605
InterPro Count	3644
KO Count assembled	3275
Not KO Count assembled	3236
Pfam Cluster Count assembled	2441
Paralogs Count	2322
COG Cluster Count assembled	2036
KEGG Count assembled	1906
TIGRfam Count assembled	1634
Enzyme Count assembled	1563
IMG Term Count	1535
MetaCyc Count assembled	1376
TIGRfam Cluster Count assembled	1213
KOG Count	1209
w/o function prediction assembled	1140
TC Count	1091
Transmembrane Count	715
Chromosomal Cassette Count	618
Signal Peptide Count	590
KOG Cluster Count	586
Biosynthetic Cluster Gene Count assembled	569
IMG Parts List Count	521

Fused Count	392
IMG Pathway Count	358
Pseudo Genes Count	111
CDS % assembled	99.19
Chromosomal Cassette Gene %	97.61
Coding Base Count %	87.42
Coding Base Count NP %	86.49
Pfam % assembled	83.27
w/ Func Pred % assembled	81.83
Not MetaCyc % assembled	78.23
COG % assembled	73.16
Not KEGG % assembled	70.16
Scaffold Count assembled	60
InterPro %	55.51
RNA Count assembled	53
KO % assembled	49.89
Not KO % assembled	49.3
tRNA Count assembled	46
Paralogs %	35.37
Horizontally Transferred Count	31
Biosynthetic Cluster Count assembled	30
KEGG % assembled	29.04
TIGRfam % assembled	24.89
Enzyme % assembled	23.81
IMG Term %	23.39
MetaCyc % assembled	20.96
KOG %	18.42
w/o function prediction % assembled	17.37

TC %	16.62
Transmembrane %	10.89
Signal Peptide %	8.99
Biosynthetic Cluster Gene % assembled	8.67
IMG Parts List %	7.94
Fused %	5.97
IMG Pathway %	5.45
rRNA Count assembled	4
Other RNA Count assembled	3
5S rRNA Count assembled	2
Pseudo Genes %	1.69
23S rRNA Count assembled	1
16S rRNA Count assembled	1
RNA % assembled	0.81
GC assembled	0.61
Horizontally Transferred %	0.47

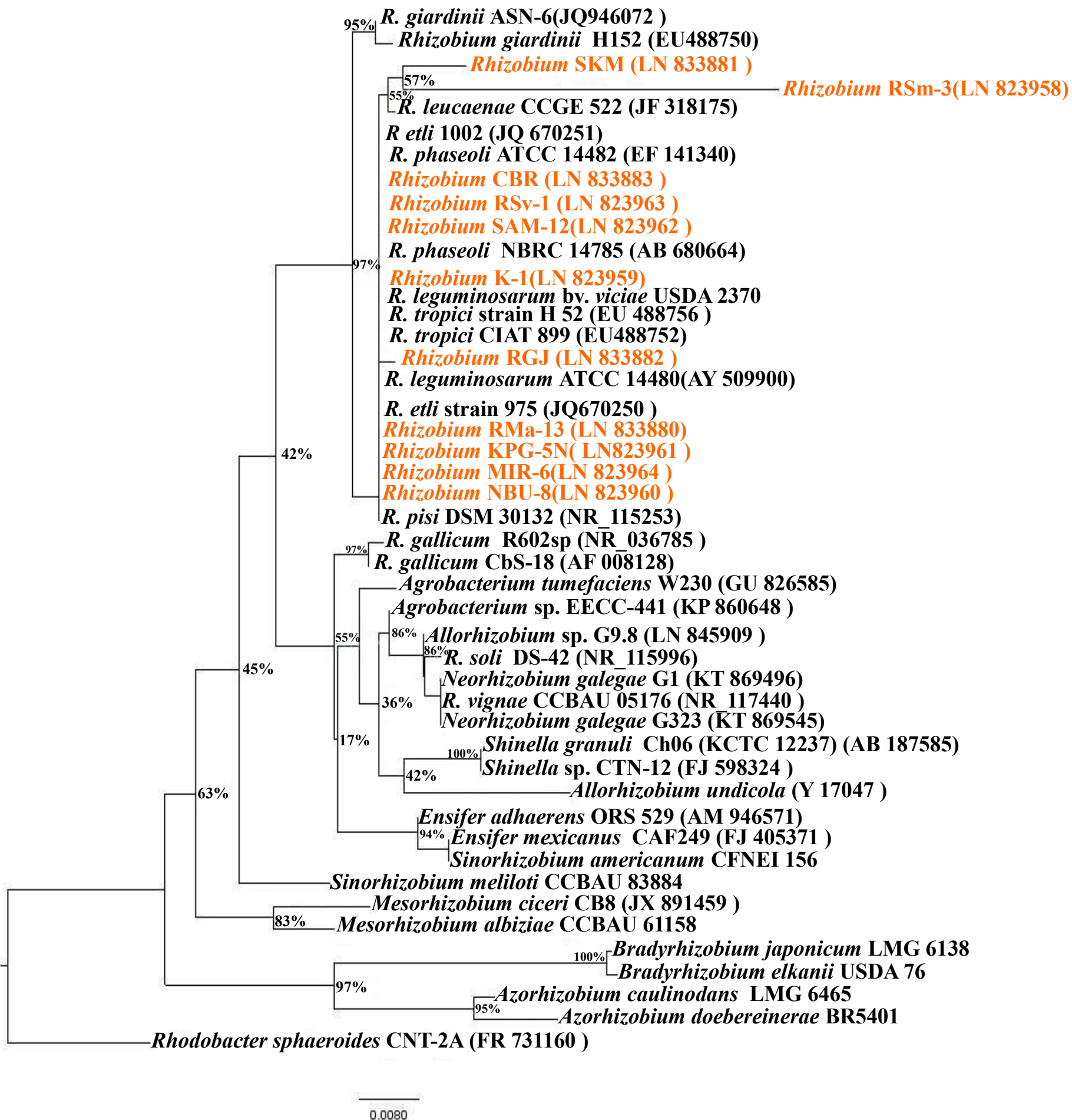


Fig SF1: Dendrogram generated by RAxML representing the relationship of the eleven *Rhizobium* strains with the standard GenBank partial sequences of rhizobial 16SrDNA .