

DATASET BRIEF

Two-dimensional proteome reference map of *Rhizobium tropici* PRF 81 reveals several symbiotic determinants and strong resemblance with agrobacteria

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Rhizobium tropici strain PRF 81 is used in commercial inoculants for common-bean crops in Brazil because of its high efficiency in nitrogen fixation and, as in other strains belonging to this species, its tolerance of environmental stresses, representing a useful biological alternative to chemical nitrogen fertilizers. In this study, a proteomic reference map of PRF 81 was obtained by two-dimensional gel electrophoresis and MALDI-TOF/TOF-TOF mass spectrometry. In total, 115 spots representing 109 different proteins were successfully identified, contributing to a better understanding of the rhizobia-legume symbiosis and supporting, at proteomics level, a strong resemblance with agrobacteria.

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Microbiology / Nitrogenase / Proteomics reference map / Symbiosis

Alternative technologies to improve agricultural productivity are necessary; however, many of them are harmful to the environment. In contrast, biological nitrogen fixation by rhizobia-legume symbiosis represents an environmentally friendly and economically sound strategy to achieve high yields [1]. *Rhizobium tropici* strain PRF 81 (= SEMIA 4080) is recognized by both its high efficiency in fixing nitrogen in association with common bean (*Phaseolus vulgaris* L.), and its high tolerance to environmental stresses [2]. These features, in addition to the higher genetic stability in comparison with other common bean rhizobia [2], resulted in this strain being broadly used in inoculants in Brazil [1]. The genome of PRF 81 [3] is now in progress (<http://www.bnf.lncc.br>), but proteomic studies are fundamental to understand gene expression. In this study, we present a two-dimensional gel electrophoresis (2DE) proteomic reference map of *R. tropici*

PRF 81, which allowed the identification of several molecular determinants related to the symbiosis and highlighted a close phylogenetic relationship with strains of *Agrobacterium* (= *Rhizobium*).

R. tropici strain PRF 81, isolated from common bean nodules in Brazil, is deposited at the “Diazotrophic and Plant Growth Promoting Bacteria Culture Collection” of Embrapa Soja. More details about the strain are given elsewhere [1, 3]. To achieve the whole-cell protein extract, the bacterium was grown in TY medium and proteins were extracted as described by Batista et al. [4], except for that 40 cycles of freezing and thawing were used.

The whole protein extract was purified with phenol and dissolved with DeStreak buffer (GE Healthcare, Uppsala, Sweden) to a final concentration of 300 µg. For 2DE, performed in triplicate, the procedure was as described before [4], modified only by the IPG-strips pH range (pH 4–7). After being stained with Coomassie Blue PhastGel™ R-350 (GE Healthcare), the gels were analyzed by Image Master 2D Platinum v 5.0 (GE Healthcare). Well-defined spots, present in all three gels were selected, excised, and processed as described before [5]. Digestion was achieved with trypsin (Gold Mass Spectrometry Grade, Promega) at 37°C overnight.

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Abbreviation: COG, Clusters of orthologous groups

Mass spectra were acquired in a MALDI-TOF-TOF Autoflex Spectrometer (Bruker Daltonics, Billerica, MA, USA), which was operated in the reflector mode for MALDI-TOF peptide mass fingerprint (PMF) and in the “LIFT” mode for MALDI-TOF-TOF in the fully manual mode, using FlexControl software v. 2.2 and processed using Flex Analysis v. 3.0 (Bruker Daltonics). The PMFs and MS/MS ion spectra generated were searched against the public database NCBI nr using Mascot software search engine v. 2.3 (<http://www.matrixscience.com/>) as previously described [4]. Identifications, available at PRIDE (<http://ebi.ac.uk/pride/>) with the experiment accession number 14817, were validated only when the MOWSE (Molecular Weight Search) score was significant, and both decoy score and false discovery rates were considered.

To improve the separation of *R. tropici* PRF 81 proteins, we employed IPG strips with a narrow pH range, 4.0–7.0. This procedure achieved better protein resolution than with a broader range, of pH 3.0–10.0 (data not shown). Among 880 spots detected on gels, 150 were randomly selected and 115 spots, representing 109 different proteins, were successfully identified and are indicated by their numbers in Fig. 1 and listed in Table S1 of Supporting Information.

Although most proteins extracted by this methodology are located in cell cytoplasm and periplasmic space, some membrane and extra cellular proteins can also be obtained. In addition, the presence of distinct spots for the same protein may be the result of posttranslational modifications, e.g., glutamine synthetase I (spots 12, 13, and 14), which in *Escherichia coli* is an example of posttranslational regulated enzymes [6].

According to the functional classification in clusters of orthologous group (COG), proteins were distributed in 17 categories, belonging to four functional groups: metabolic functions (C, E, F, G, H, I, P); cellular processes and signaling (D, T, O, M, N); information storage and processing (J, K, L); and poorly characterized proteins (R, S) (Fig. 2). Six proteins were classified as hypothetical/conserved hypothetical and only four did not fit in any of the categories, being assigned as “not in COG.”

Besides its ability to establish a symbiotic partnership with common bean, *R. tropici* PRF 81 is also characterized by high competitiveness against indigenous rhizobial populations [1, 7, 8]. This feature is directly related to metabolic plasticity shown in vitro by this species [9], which can assimilate a wide range of carbon and nitrogen sources by the Entner–Doudoroff pathway, glycolysis, and the tricarboxylic acid cycle [10]. Our proteomic study confirmed metabolic diversity, because more than 51% of identified proteins were associated with metabolic functions. The most representative category, grouping 26% of the metabolic proteins, was that of amino acid metabolism, that can directly influence nitrogen fixation in the legume-rhizobia symbiosis [9].

Nitrogen-fixing bacteria also have high requirements for iron, essential for the activity of many enzymes, including those involved in nitrogen fixation—such as nitrogenase, cytochromes, and ferredoxin—which contain Fe as their prosthetic groups [11]. Like other microbes, those belonging to rhizobial genera use various strategies to acquire iron, and one of the most well studied is the TonB-dependent receptor (TBDR), identified in our study [12].

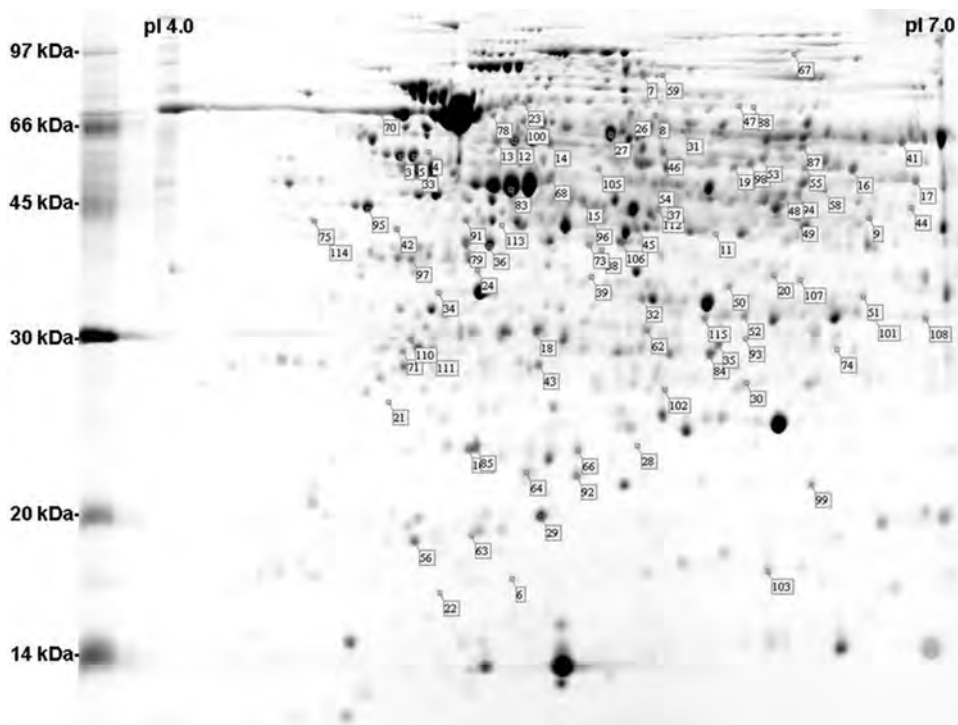


Figure 1. 2DE protein profile of *Rhizobium tropici* strain PRF 81 whole cell extract. More information about identified proteins assigned is available in Supporting Information Table S1.

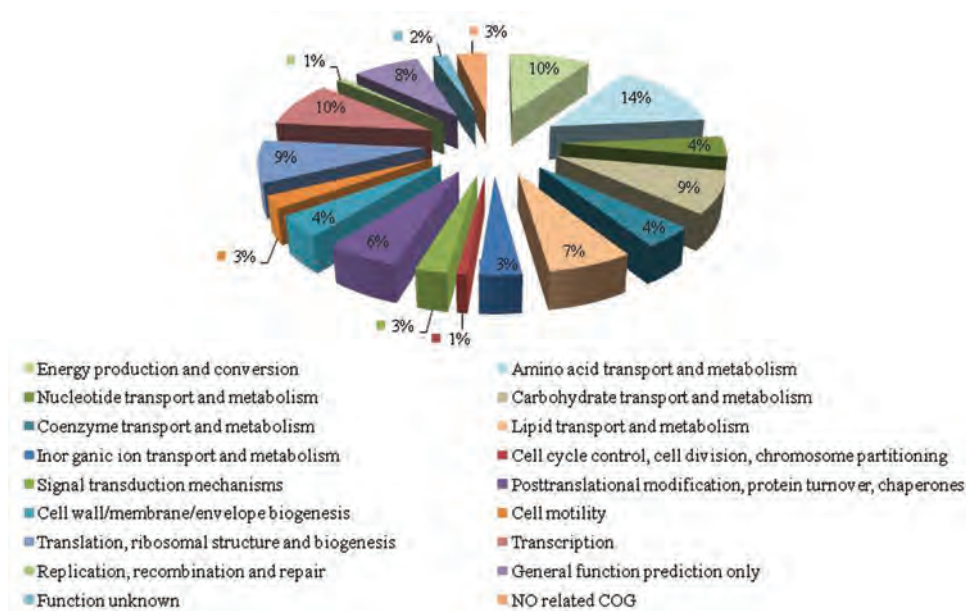


Figure 2. Distribution of *R. tropici* PRF 81 proteins identified by MS into COG categories.

Biological nitrogen fixation process involves several intricate steps, initiated by the exchange of molecular signals between the symbionts. Several proteins related to these steps encoded by PRF 81 were evidenced among the 19 proteins distributed in the different categories of the cellular process and signaling functional group, e.g., ChvI. This protein is the response regulator of a two-component regulatory system that, in response to specific plant signals, regulates positively the transcription of *exo* genes, encoding enzymes for succinoglycan exopolysaccharide (EPSI), critical for the establishment of the symbiosis [13].

Present in many bacteria, and now reported in PRF 81, the twin-arginine translocation (Tat) system transports folded proteins across the cytoplasmic membrane, including symbiosis and pathogenicity determinants [14]. In addition, the essential role of the Tat system for the rhizobia-legume symbiosis has been highlighted [15].

Outer membrane lipoprotein (Omp), a peptidoglycan-associated protein, was also identified in our study. Well characterized in *E. coli*, it has a multifunctional role, affecting cell morphology, virulence, and host-pathogen interaction [16]. Omp also acts as a host cell adhesion factor, and differential expression in *Gluconacetobacter diazotrophicus* co-cultivated with sugarcane plantlets has been reported [17], suggesting that the protein acts in the plant-bacterium interaction.

Also noteworthy are two proteins expressed in PRF 81, VirD4 and ATP-dependent Clp protease (ClpA). VirD4 is a member of the type IV secretion system (T4SS), which in *Agrobacterium tumefaciens* participates in DNA processing and transfer reactions [18]. The second, ClpA, is part of the ClpAP protease system, whose importance has been reported in the regulation of bacterial polysaccharide production [19], stress tolerance, cell division, and motility [20].

The transcription factors have fundamental roles in controlling metabolic pathways, because they regulate gene expression levels [17]. In our study, nine proteins were assigned to this functional category. Among these, we report the expression of a protein belonging to the LysR family of transcriptional regulators, known to act in the establishment of the symbiosis *S. meliloti*-alfalfa (*Medicago sativa* L.), probably through the regulation of *nod* gene expression [21]; a similar role might be found in *R. tropici*.

Several antioxidant factors were identified in our study, among them alkyl hydroperoxide reductase that plays an important role in cell defense against oxidative stress, because it uses H_2O_2 as substrate [22]. Reactive oxygen species (ROS) are by-products of normal metabolic processes and at high levels were first thought to be lethal for cells. However, in both symbiotic and pathogenic relations, transient production of ROS, detected in early events of plant–microorganism interactions, may be considered as specific signals during the interaction process [23]; therefore, it should be overcome by the microsymbionts. Other two proteins encoded by PRF 81 that show antioxidant activity were the bacterioferritin (Bfr) and bacterioferritin comigratory (Bcp) proteins.

Species of rhizobia and agrobacteria are closely related, and the inclusion of *Agrobacterium* species in the genus *Rhizobium* has been proposed [24]. Consistent with this, *R. tropici* strain PRF 81 has demonstrated high resemblance in many genes [3], and now also of many proteins to those of strains belonging to the genus *Agrobacterium*.

Among the proteins resembling agrobacteria, it is worth mentioning the LysR family transcriptional regulator. In agrobacteria they are related to *chvE* gene expression, the product of which induces a set of bacterial virulence (*vir*) genes that are necessary for plant infection [25]. Another pro-

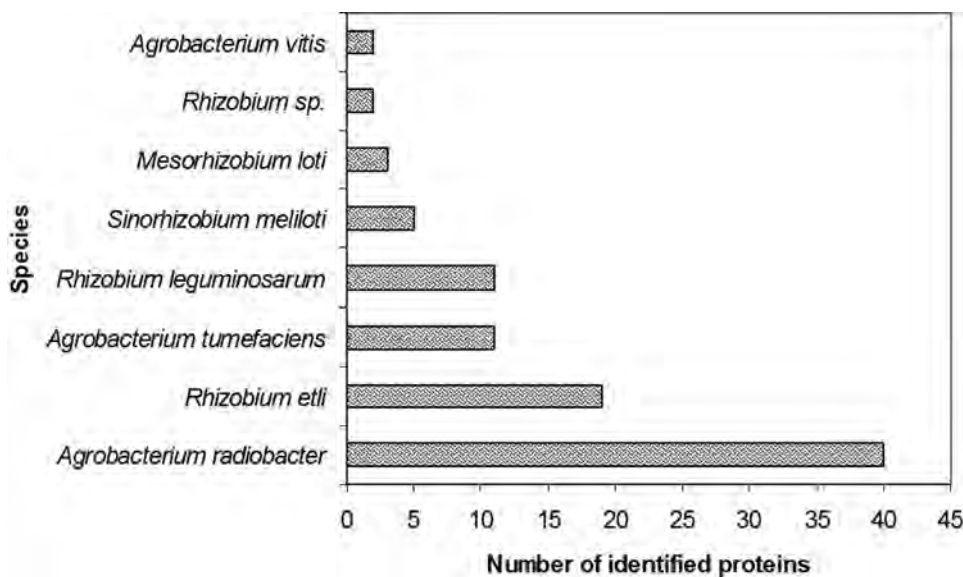


Figure 3. Amount of proteins identified with higher score for each different species. Species with only one protein identified are not shown.

tein now identified at proteomic level, and previously identified in the draft genome of PRF 81 [3] was the VirD4, playing a role in the DNA transfer from agrobacteria to the host plant [26].

Noteworthy is also that most of the PRF 81 proteins matched – with high scores – *Agrobacterium* proteins (Fig. 3), even though the number of peptide sequences of *Rhizobium* in the NCBI database is about three times higher than that of *Agrobacterium*. Therefore, our data strengthen previous observations that *R. tropici* and *Agrobacterium* share a high level of resemblance.

In this paper, we present a 2D reference map of *R. tropici* strain PRF 81, an agronomically important nitrogen-fixing microsymbiont that is poorly characterized at the molecular level. Various molecular determinants of the establishment of symbiosis between *R. tropici* and common bean are highlighted, contributing to a better understanding of the symbiosis. We have also reported important proteins related to cell protection against several stresses. Finally, we have shown that *R. tropici* presents high resemblance with agrobacteria, as previously observed at the genomic level [3], and now for the first time confirmed in a proteomic study.

The spectrometry dataset is available at PRIDE (<http://ebi.ac.uk/pride/>), with the accession number 14817.

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The authors have declared no conflict of interest.

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Table 1: Identified proteins of *Rhizobium tropici* PRF 81 whole cell extracts. Matched peptides masses and MS/MS combined results are available in PRIDE (<http://ebi.ac.uk/pride/>) under the experiment accession number 14817.

Spot ID	NCBI ID	Gene	Protein description	Organism	*T. pI	**E. pI	T. mass	E. mass	Cellular location
Metabolism									
C - Energy production and conversion									
1	gi 222087461	<i>sucC</i>	Succinyl-CoA synthetase beta subunit protein	<i>Agrobacterium radiobacter</i>	4,98	4,96	42028	46000	Cytoplasmic
2	gi 86359524	<i>acnA</i>	Aconitate hydratase	<i>Rhizobium etli</i>	5,48	5,69	97180	98000	Cytoplasmic
3	gi 116254139	<i>atpD</i>	F0F1 ATP synthase subunit beta	<i>Rhizobium leguminosarum</i>	5,03	4,88	50885	56000	Cytoplasmic
4	gi 15966787	<i>atpD</i>	F0F1 ATP synthase subunit beta	<i>Sinorhizobium meliloti</i>	5,35	4,99	53250	57000	Cytoplasmic
5	gi 227823626	<i>atpD</i>	ATP synthase F1, beta subunit	<i>Rhizobium sp.</i>	5,27	4,94	53408	56000	Cytoplasmic
6	gi 86359450	<i>atpC</i>	F0F1 ATP synthase subunit epsilon	<i>Rhizobium etli</i>	5,21	5,32	15097	17000	Cytoplasmic
7	gi 222085583		NADH-quinone oxidoreductase, chain G	<i>Agrobacterium radiobacter</i>	5,44	5,83	75528	85000	Cytoplasmic
8	gi 222086219	<i>fumB</i>	Fumarate hydratase class I protein	<i>Agrobacterium radiobacter</i>	6,01	5,88	59014	69000	Cytoplasmic
9	gi 13471013		Glutathione dependent formaldehyde dehydrogenase	<i>Mesorhizobium loti</i>	12	6,71	40481	43000	Cytoplasmic
10	gi 222087510	<i>acnA</i>	Aconitate hydratase 1	<i>Agrobacterium radiobacter</i>	5,23	5,76	98443	97000	Cytoplasmic
11	gi 222086991		Oxidoreductase protein	<i>Agrobacterium radiobacter</i>	5,66	6,11	36032	41000	Cytoplasmic
E- Amino acid transport and metabolism									
12	gi 1245379	<i>glnA</i>	Glutamine synthetase I	<i>Sinorhizobium meliloti</i>	5,2	5,33	52287	61000	Cytoplasmic
13	gi 153009417	<i>glnA</i>	Glutamine synthetase I	<i>Ochrobactrum anthropi</i>	5,38	5,26	52469	61000	Cytoplasmic
14	gi 6601379	<i>glnA</i>	Glutamine synthetase	<i>Rhizobium leguminosarum</i>	6,07	5,47	34686	60000	Cytoplasmic
15	gi 222087908	<i>asd</i>	Aspartate-B-semialdehyde dehydrogenase protein	<i>Agrobacterium radiobacter</i>	5,46	5,59	37925	45000	Cytoplasmic
16	gi 209551290		Argininosuccinate synthase	<i>Rhizobium leguminosarum</i>	6,00	6,64	45203	53000	Cytoplasmic
17	gi 86357146	<i>glyA</i>	Serine hydroxymethyltransferase	<i>Rhizobium etli</i>	6,29	6,89	46740	50000	Cytoplasmic
18	gi 15887731	<i>argB</i>	Acetylglutamate kinase	<i>Agrobacterium tumefaciens</i>	5,16	5,41	31083	30000	Cytoplasmic
19	gi 241204468		Homoserine dehydrogenase	<i>Rhizobium leguminosarum</i>	5,39	6,18	47035	53000	Cytoplasmic
20	gi 15964271	<i>argF1</i>	Ornithine carbamoyltransferase	<i>Sinorhizobium meliloti</i>	6,09	6,34	33643	36000	Cytoplasmic
21	gi 86359669	<i>leuD</i>	Isopropylmalate isomerase small subunit	<i>Rhizobium etli</i>	5,02	4,83	22397	26000	Cytoplasmic
22	gi 22208582	<i>gloA</i>	Lactoylglutathione lyase	<i>Agrobacterium radiobacter</i>	5,03	5,03	16541	16000	Cytoplasmic
23	gi 116253255	<i>leuA</i>	2-Isopropylmalate synthase	<i>Rhizobium leguminosarum</i>	5,15	5,35	62565	73000	Cytoplasmic

24	gi 222087414	<i>rocF</i>	Arginase	<i>Agrobacterium radiobacter</i>	5,3	5,17	33045	36000	Cytoplasmic
25	gi 16264577	<i>hutU</i>	Urocanate hydratase	<i>Sinorhizobium meliloti</i>	5,94	5,87	61859	86000	Cytoplasmic
26	gi 190893841		Dipeptide ABC transporter	<i>Rhizobium etli</i>	5,23	5,78	60139	70000	Periplasmic
27	gi 222084939		Dipeptide ABC transporter	<i>Agrobacterium radiobacter</i>	5,21	5,7	59112	62000	Periplasmic
F- Nucleotide transport and metabolism									
28	gi 159184890	<i>gtp</i>	Xanthine-guanine phosphoribosyltransferase	<i>Agrobacterium tumefaciens</i>	5,35	5,8	18487	23000	Cytoplasmic
29	gi 116251346	<i>ndk</i>	Nucleoside diphosphate kinase	<i>Rhizobium leguminosarum</i>	5,65	5,43	15314	20000	Cytoplasmic
30	gi 190889872	<i>upp</i>	Uracil phosphoribosyltransferase protein	<i>Rhizobium etli</i>	5,98	6,23	23305	27000	Cytoplasmic
31	gi 209551875	<i>guaA</i>	GMP synthase	<i>Rhizobium leguminosarum</i>	5,56	5,98	57742	64000	Cytoplasmic
32	gi 86146888	<i>pyrH</i>	Uridylate kinase	<i>Vibrio sp.</i>	5,08	5,82	26284	33000	Cytoplasmic
G - Carbohydrate transport and metabolism									
33	gi 222085874	<i>eno</i>	Phosphopyruvate hydratase	<i>Agrobacterium radiobacter</i>	4,84	4,95	45120	53000	Cytoplasmic
34	gi 282887091		Alpha amylase catalytic region	<i>Burkholderia sp.</i>	6,26	5,03	64245	34000	Cytoplasmic
35	gi 241206422	<i>talB</i>	Transaldolase	<i>Rhizobium leguminosarum</i>	5,32	6,12	35091	29000	Cytoplasmic
36	gi 91975363		Polysaccharide deacetylase	<i>Rhodopseudomonas palustris</i>	8,31	5,23	39900	39000	Cytoplasmic
37	gi 222086498		Sugar ABC transporter	<i>Agrobacterium radiobacter</i>	5,39	5,91	46099	45000	Periplasmic
38	gi 86356131	<i>frk</i>	Fructokinase protein	<i>Rhizobium etli</i>	5,22	5,66	32940	39000	Cytoplasmic
39	gi 222084754	<i>frcB</i>	Fructose ABC transporter	<i>Agrobacterium radiobacter</i>	5,64	5,62	35424	36000	Periplasmic
40	gi 86359086	<i>tkt</i>	Transketolase	<i>Rhizobium etli</i>	6,07	6,49	70055	85000	Cytoplasmic
41	gi 222084912	<i>zwf</i>	Glucose-6-phosphate 1-dehydrogenase	<i>Agrobacterium radiobacter</i>	6,13	6,84	55064	60000	Cytoplasmic
42	gi 222084346		Sugar kinase protein	<i>Agrobacterium radiobacter</i>	4,7	4,87	36089	41000	Cytoplasmic
H - Coenzyme transport and metabolism									
43	gi 296105270		Biotin--protein ligase	<i>Enterobacter cloacae</i>	5,23	5,42	35255	28000	Cytoplasmic
44	gi 159184833		Coproporphyrinogen III oxidase	<i>Agrobacterium tumefaciens</i>	6,88	6,67	50241	44000	Cytoplasmic
45	gi 148265868		Phosphomethylpyrimidine kinase	<i>Geobacter uraniireducens</i>	5,8	5,81	52613	41000	Cytoplasmic
46	gi 209551535		S-Adenosyl-L-homocysteine hydrolase	<i>Rhizobium leguminosarum</i>	5,49	5,9	51007	57000	Cytoplasmic
47	gi 240850082	<i>dxs</i>	1-Deoxy-D-xylulose-5-phosphate synthase Dxs	<i>Bartonella grahamii</i>	6,44	6,2	69040	73000	Cytoplasmic
I – Lipid transport and metabolism									
48	gi 159186213	<i>pcaF</i>	Beta-ketoadipyl CoA thiolase	<i>Agrobacterium tumefaciens</i>	5,51	6,37	41850	46000	Cytoplasmic
49	gi 261823376	<i>pldB</i>	Lysophospholipase	<i>Pectobacterium wasabiae</i>	6,43	8,22	39779	43000	Inner membrane

50	gi 197122598		Acyl-CoA dehydrogenase domain protein	<i>Anaeromyxobacter sp.</i>	5,7	6,17	40084	35000	Cytoplasmic
51	gi 15966435		Acetyl-CoA carboxylase carboxyltransferase subunit alpha	<i>Sinorhizobium meliloti</i>	6,04	6,68	34716	34000	Cytoplasmic
52	gi 222085109	<i>fabI2</i>	Enoyl-(acyl-carrier-protein) reductase (NADH) protein	<i>Agrobacterium radiobacter</i>	5,7	6,22	29153	32000	Cytoplasmic
53	gi 222085639		Acetyl-CoA carboxylase, biotin carboxylase	<i>Agrobacterium radiobacter</i>	5,64	6,29	49554	55000	Cytoplasmic
54	gi 13474638		3-Oxoacyl-(acyl carrier protein) synthase I	<i>Mesorhizobium loti</i>	5,5	5,87	43201	49000	Cytoplasmic
55	gi 222085444	<i>fabF1</i>	3-Oxoacyl-(acyl-carrier protein) synthase II	<i>Agrobacterium radiobacter</i>	5,73	6,46	44384	53000	Cytoplasmic
P - Inorganic ion transport and metabolism									
56	gi 222087891	<i>bfr</i>	Bacterioferritin	<i>Agrobacterium radiobacter</i>	4,81	4,94	16860	19000	Cytoplasmic
57	gi 87199081		TonB-dependent receptor	<i>Novosphingobium aromaticivorans</i>	5,82	5,01	87810	75000	Extra Cellular
58	gi 238795940		Glucans biosynthesis protein	<i>Yersinia mollaretii</i>	6,41	6,53	58198	48000	Periplasmic
59	gi 46580895		ABC transporter, ATP-binding protein	<i>Desulfovibrio vulgaris</i>	6,04	5,9	67370	86000	Cytoplasmic
Cellular processes and signaling									
D - Cell cycle control, cell division, chromosome partitioning									
60	gi 222086436	<i>ftsZ2</i>	Cell division protein FtsZ	<i>Agrobacterium radiobacter</i>	5,21	5,39	63014	81000	Cytoplasmic
T - Signal transduction mechanisms									
61	gi 222087232	<i>prkA</i>	Serine protein kinase protein	<i>Agrobacterium radiobacter</i>	5,42	5,69	74417	84000	Cytoplasmic
62	gi 159184131	<i>chvI</i>	Two component response regulator	<i>Agrobacterium tumefaciens</i>	5,56	5,85	27253	30000	Cytoplasmic
63	gi 209549558		Transcriptional regulator, TraR/DksA family	<i>Rhizobium leguminosarum</i>	5,15	5,16	16338	19000	Cytoplasmic
O - Posttranslational modification, protein turnover, chaperones									
64	gi 118590060	<i>bcp</i>	Bacterioferritin comigratory protein	<i>Stappia aggregata</i>	5,63	5,37	16749	22000	Cytoplasmic
65	gi 222085003	<i>groE_L</i>	Chaperonin GroEL	<i>Agrobacterium radiobacter</i>	5,03	5,11	57836	69000	Cytoplasmic
66	gi 53716307	<i>ahpC</i>	Alkyl hydroperoxide reductase, subunit c	<i>Burkholderia mallei</i>	5,17	5,57	20749	23000	Cytoplasmic
67	gi 222085851	<i>clpA</i>	ATP-dependent Clp protease	<i>Agrobacterium radiobacter</i>	5,93	6,41	92748	97000	Cytoplasmic
68	gi 15888589	<i>clpX</i>	ATP-dependent protease ATP-binding subunit	<i>Agrobacterium tumefaciens</i>	5,3	5,47	47266	49000	Cytoplasmic
69	gi 15888590	<i>lon</i>	ATP-dependent protease LA	<i>Agrobacterium tumefaciens</i>	5,63	6,36	88751	100000	Cytoplasmic
70	gi 222085803	<i>tig</i>	Trigger factor protein	<i>Agrobacterium radiobacter</i>	4,85	4,78	54566	72000	Cytoplasmic

M - Cell wall/membrane/envelope biogenesis

71	gi 86359655	Putative metalloendopeptidase protein	<i>Rhizobium etli</i>	5,36	4,89	49514	29000	Periplasmic
72	gi 222085864	<i>omp1</i> Outer membrane lipoprotein	<i>Agrobacterium radiobacter</i>	5,26	5,76	84589	79000	Outer Membrane
73	gi 222087790	<i>galE</i> UDP-glucose 4-epimerase	<i>Agrobacterium radiobacter</i>	5,39	5,62	35969	39000	Cytoplasmic
74	gi 222084436	Hypothetical protein Arad_0321	<i>Agrobacterium radiobacter</i>	6,34	6,58	31726	29000	Cytoplasmic
75	gi 103487746	Twin-arginine translocation pathway signal	<i>Agrobacterium radiobacter</i>	8,63	4,54	45113	42000	Periplasmic

N - Cell motility

76	gi 222087318	<i>secA</i> Protein-export translocase protein	<i>Agrobacterium radiobacter</i>	5,13	5,37	102186	104000	Cytoplasmic
77	gi 13473495	GTP-binding protein TypA	<i>Mesorhizobium loti</i>	5,57	5,47	67143	85000	Cytoplasmic
78	gi 18033179	<i>virD4</i> VirD4	<i>Agrobacterium tumefaciens</i>	6,82	5,24	73380	69000	Cytoplasmic

Information storage and processing**J - Translation, ribosomal structure and biogenesis**

79	gi 222085858	<i>tsf</i> Translation elongation factor Ts	<i>Agrobacterium radiobacter</i>	5,15	5,14	32268	40000	Cytoplasmic
80	gi 227821753	<i>fusA</i> Elongation factor G	<i>Rhizobium sp.</i>	5,17	5,3	77966	89000	Cytoplasmic
81	gi 86355771	<i>pnp</i> Polynucleotide phosphorylase/polyadenylase	<i>Rhizobium etli</i>	5,2	5,19	77491	89000	Cytoplasmic
82	gi 294624706	<i>infB</i> Translation initiation factor IF-2	<i>Xanthomonas fuscans</i>	5,89	5,76	83626	75000	Cytoplasmic
83	gi 218672404	Elongation factor EF-Tu protein	<i>Rhizobium etli</i>	4,87	5,31	31884	48000	Cytoplasmic
84	gi 222086595	<i>rplY</i> Ribosomal protein L25, Ctc-form	<i>Agrobacterium radiobacter</i>	5,6	6,09	21953	29000	Cytoplasmic
85	gi 86357075	<i>rplI</i> 50S ribosomal protein L9	<i>Rhizobium etli</i>	4,82	5,18	21057	23000	Cytoplasmic
86	gi 15887437	<i>pnpA</i> Polynucleotide phosphorylase/polyadenylase	<i>Agrobacterium tumefaciens</i> <i>Agrobacterium radiobacter</i>	5,21	5,23	77366	89000	Cytoplasmic
87	gi 222085595	<i>proS</i> Prolyl-tRNA synthetase protein	<i>Sphingopyxis alaskensis</i>	5,65	6,45	53483	59000	Cytoplasmic
88	gi 222086050	<i>thrS</i> Threonyl-tRNA synthetase	<i>Agrobacterium radiobacter</i>	5,59	6,25	75209	72000	Cytoplasmic

K - Transcription

89	gi 222081230	Transcriptional regulator protein	<i>Agrobacterium radiobacter</i>	6,38	5,6	98220	98000	Cytoplasmic
90	gi 190895600	Transcriptional regulator AraC family	<i>Rhizobium etli</i>	6,91	5,42	42937	85000	Cytoplasmic
91	gi 222106466	Transcriptional regulator ROK family	<i>Agrobacterium vitis</i>	7,03	5,14	41156	42000	Cytoplasmic
92	gi 222082875	Transcriptional regulator MarR family	<i>Agrobacterium radiobacter</i>	5,46	5,57	18141	22000	Cytoplasmic

93	gi 222109081	LysR family transcriptional regulator	<i>Agrobacterium radiobacter</i>	6,75	5,23	32302	30000	Cytoplasmic
94	gi 218662071	<i>hrcA</i> Heat-inducible transcription repressor	<i>Rhizobium etli</i>	5,05	6,43	36513	46000	Cytoplasmic
95	gi 86357329	<i>rpoA</i> DNA-directed RNA polymerase subunit alpha	<i>Rhizobium etli</i>	4,75	4,76	37330	44000	Cytoplasmic
96	gi 167836822	<i>nusA</i> Transcription elongation factor NusA	<i>Burkholderia thailandensis</i>	4,59	5,63	54873	42000	Cytoplasmic
97	gi 15890078	<i>rho</i> Transcription termination factor Rho	<i>Agrobacterium tumefaciens</i>	5,87	4,93	47017	38000	Cytoplasmic
98	gi 86359702	<i>rho</i> Transcription termination factor Rho	<i>Rhizobium etli</i>	5,86	6,25	47058	54000	Cytoplasmic
99	gi 86357292	<i>nusG</i> Transcription antitermination protein NusG	<i>Rhizobium etli</i>	6,01	6,48	20024	21000	Cytoplasmic
L - Replication, recombination and repair								
100	gi 222084927	ATP-dependent RNA helicase protein	<i>Agrobacterium radiobacter</i>	9,17	5,36	69955	67000	Cytoplasmic
Poorly characterized								
R - General function prediction only								
101	gi 222082138	<i>cpo</i> Chloride peroxidase protein	<i>Agrobacterium radiobacter</i>	7,88	6,37	34965	32000	Periplasmic
102	gi 186472508	<i>wrbA</i> Flavoprotein WrbA	<i>Burkholderia phymatum</i>	6,19	5,91	20930	26000	Cytoplasmic
103	gi 170699364	NADPH-dependent FMN reductase	<i>Burkholderia ambifaria</i>	6,71	6,31	8539	17000	Periplasmic
104	gi 194431754	<i>dkgA</i> 2,5-Diketo-d-gluconic acid reductase A	<i>Shigella dysenteriae</i>	6,22	5,15	19399	23000	Cytoplasmic
105	gi 222085370	Ferredoxin reductase protein	<i>Agrobacterium radiobacter</i>	5,88	5,65	43777	53000	Cytoplasmic
106	gi 222087374	<i>cobS</i> Cobalamin synthase protein	<i>Agrobacterium radiobacter</i>	5,62	5,74	36882	40000	Cytoplasmic
107	gi 159184816	Hypothetical protein Atu1564	<i>Agrobacterium tumefaciens</i>	5,95	5,68	32803	35000	Cytoplasmic
108	gi 222087548	Oxidoreductase protein	<i>Agrobacterium radiobacter</i>	6,72	6,93	30503	31000	Cytoplasmic
109	gi 190892610	Probable ABC transporter, ATP-binding Protein	<i>Rhizobium etli</i>	5,34	5,62	61173	74000	Cytoplasmic
S - Function unknown								
110	gi 86359066	Hypothetical protein RHE_CH03475	<i>Rhizobium etli</i>	4,84	4,92	26773	30000	Cytoplasmic
111	gi 222149801	Hypothetical protein Avi_3814	<i>Agrobacterium vitis</i>	5,03	5,01	24632	29000	Periplasmic
NO related COG								
112	gi 209547526	Hypothetical protein Rleg2_5527	<i>Rhizobium leguminosarum</i>	6,02	5,89	33584	44000	Cytoplasmic
113	gi 254511108	TPR domain protein	<i>Rhodobacteraceae bacterium</i>	5,03	5,28	62677	42000	Cytoplasmic
114	gi 262377567	Conserved hypothetical protein	<i>Acinetobacter lwoffii</i>	4,95	4,59	37453	40000	Cytoplasmic
115	gi 222087920	Hypothetical protein Arad_4933	<i>Agrobacterium radiobacter</i>	5,89	6,07	26112	31000	Cytoplasmic

*Theoretical and **Experimental.