

Bacterial IAA-delivery into Medicago Root Nodules Triggers a Balanced Stimulation of C and N Metabolism Leading to Biomass Increase

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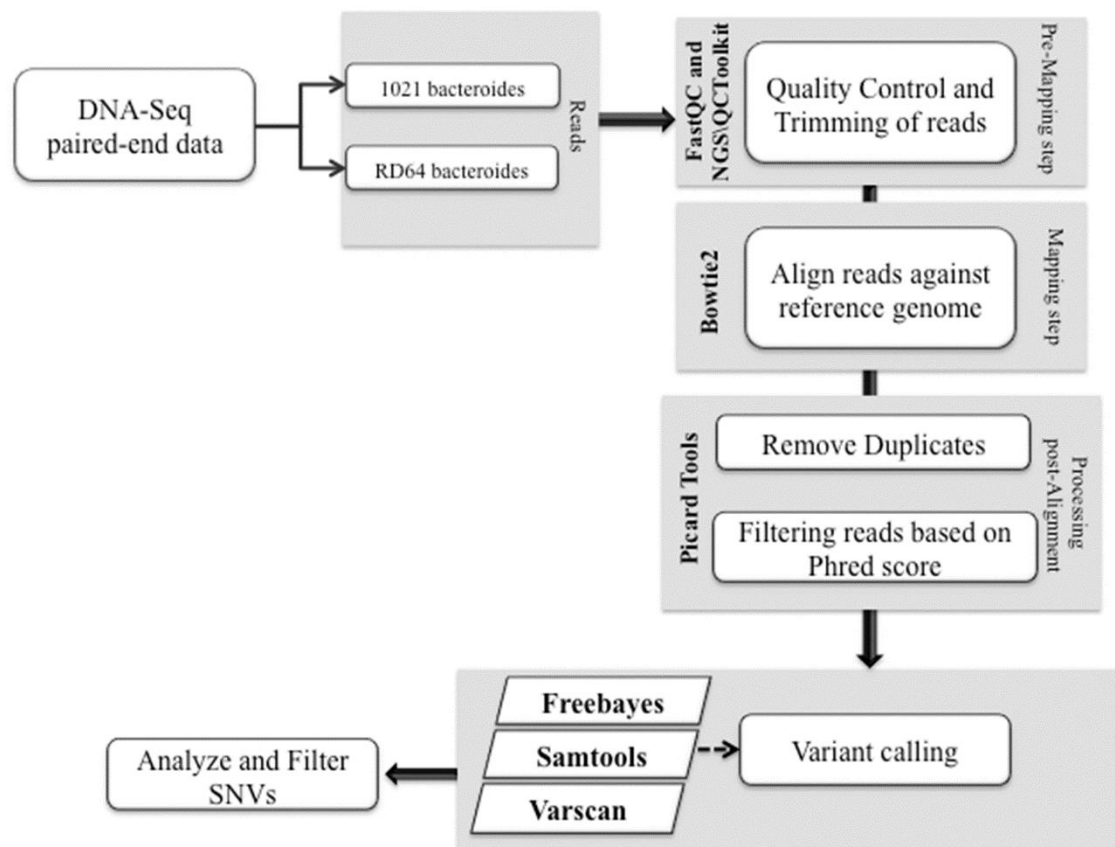


Figure S1. Workflow for the sequencing of *Ensifer meliloti* 1021 and RD64 bacteroids. The bacteroids of both strains were sequenced by a HiSeq2000 Illumina platform, and the resulting short sequencing reads mapped onto the reference genome downloaded from NCBI (http://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/Sinorhizobium_meliloti_1021_uid57603/). The variations between each sample and the reference genome were then identified. Randomly selected mutations were experimentally validated by PCR analysis.

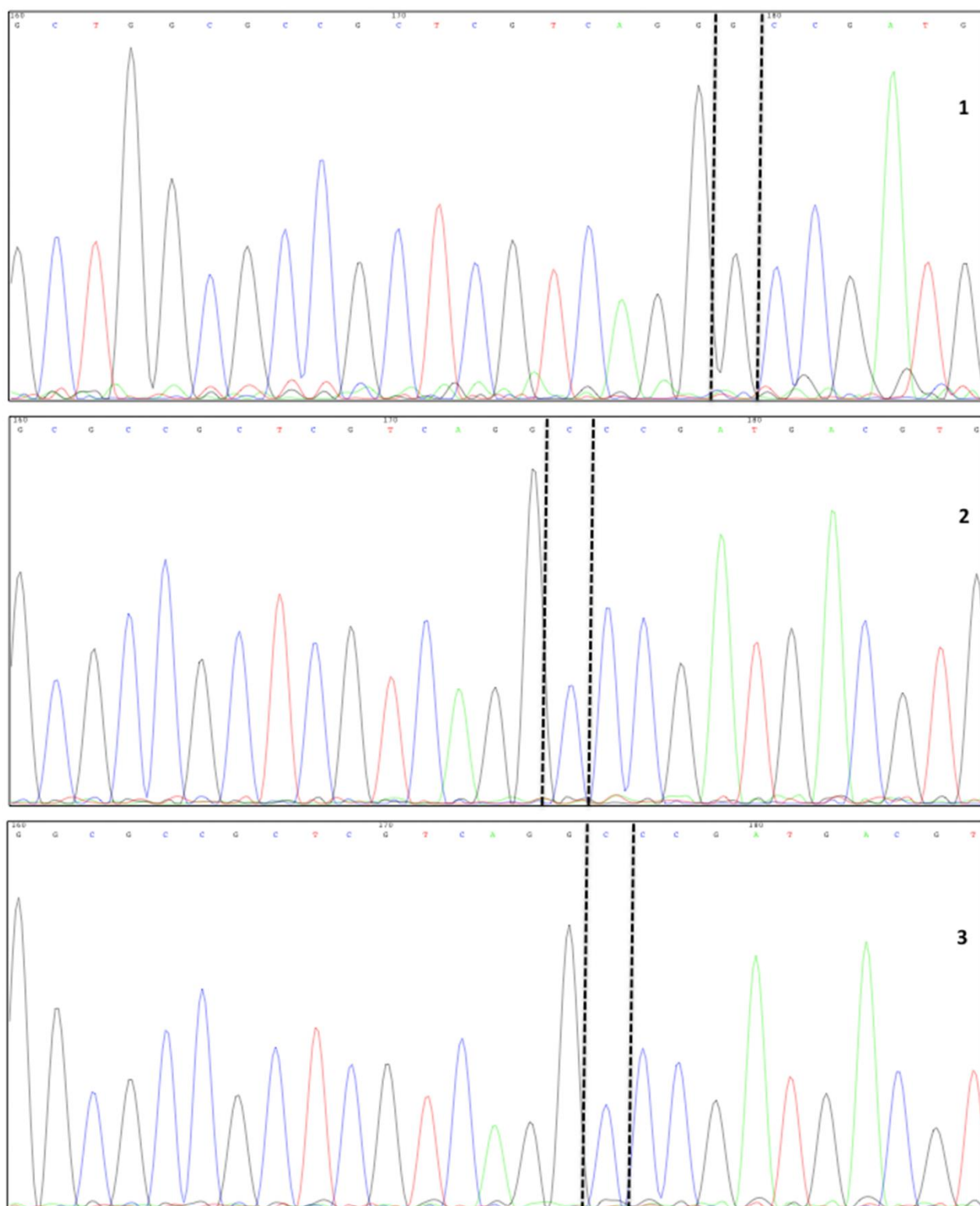


Figure S2. Electropherograms of PCR products obtained for one representative mutation selected for *Ensifer meliloti* 1021 bacteroids. The chromatograms were obtained from three biological replicates (1-3) and the dominant peaks in the sequencing traces which indicate the mutation located in the Sma0233 gene are highlighted with two dashed lines.

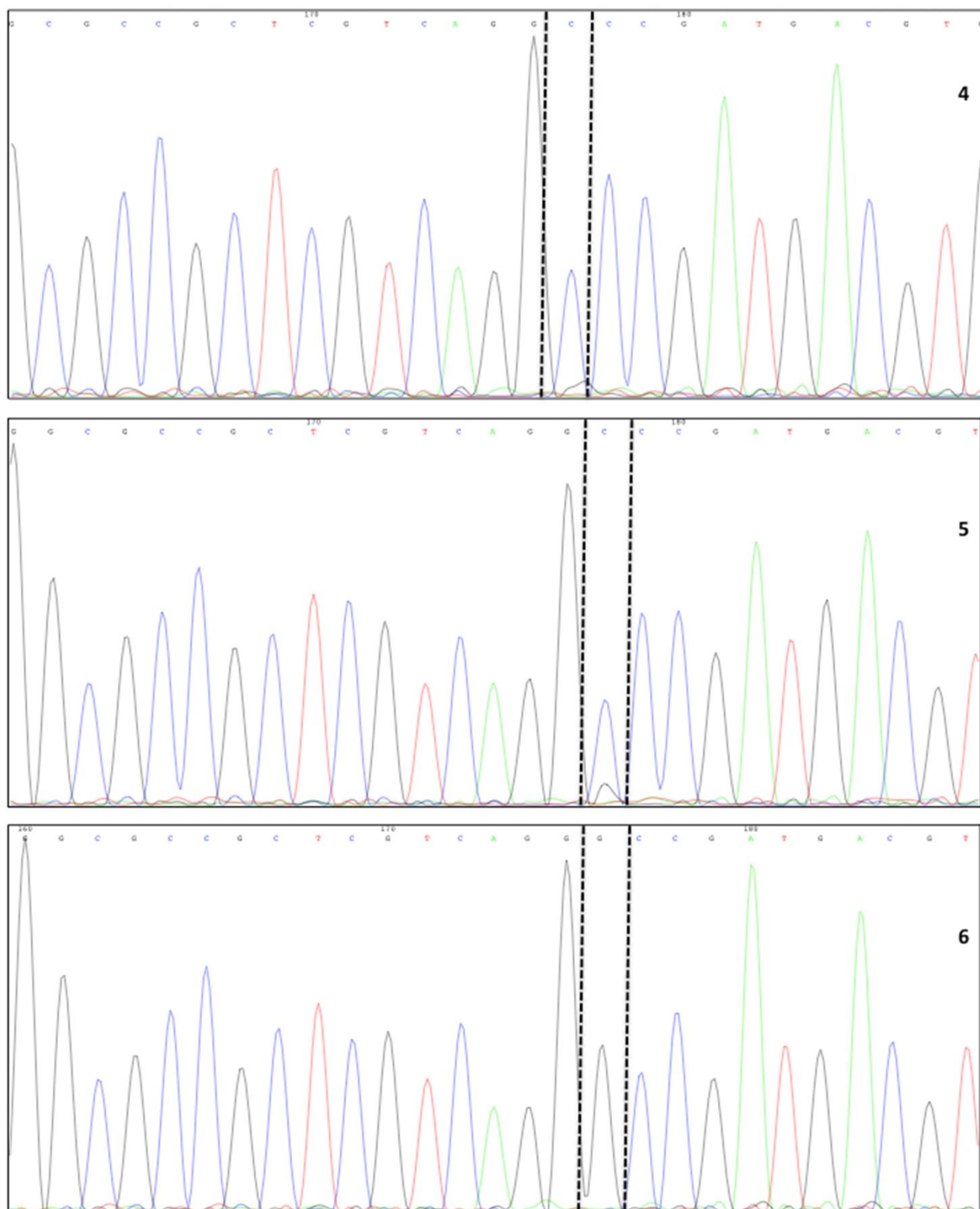


Figure S3. Electropherograms of PCR products obtained for one representative mutation selected for *Ensifer meliloti* RD64 bacteroids. The chromatograms were obtained from three biological replicates (4-6) and the dominant peaks in the sequencing traces which indicate the mutation located in the *Sma0233* gene are highlighted with two dashed lines.

Table S1. Overview of the parameters used for single nucleotide variants (SNVs) and small insertion and deletion (INDEL) calling.

Tools	Parameters
Bowtie2	-p 12 --no-discordant -N 0
VarScan	--min-coverage 30 --min-reads2 15 --min-avg-qual 25 --p-value 0.01 --min-var-freq 0.03 --output-vcf
Freebayes	--min-coverage 30 -P 0.99 -C 15 -p 1 -q 25
Samtools	varFilter -Q25
NGSQCToolkit	-q 20 -n 25

Table S2. Primers designed using Primer3 software (<http://primer3.sourceforge.net/>) and used in PCR amplifications for Illumina sequence confirmation.

Gene ID	Forward (5'→3')	Reverse (5'→3')
<i>SMa0233</i>	AGTTGCACCAGGGCAATATC	CCAGGGAATGTGGAGAAAGA
<i>SMa0461</i>	GCAAGAAGCAGACAGTGGTG	GGCTTATGCTCTTCGATTGC
<i>SMa0673</i>	GACTGGCACATCGAAGATGA	GTACGAAGCCCTCGTCTCTG
<i>SMa0745</i>	CCAAACCGATATGGCGTAAT	GCTTTTCCTTAGCGGTGTCC
<i>SMa0785</i>	CGGTGAGGCTTTTACCTTGA	ATACAGGTAGCGGTGGTTGG
<i>SMa1315</i>	AAGCATCTCCGCGACAAG	ATGACGCGATCCGAGTAGAT
<i>SMa2061</i>	GCAAGAAGCAGACAGTGGTG	GGCTTATGCTCTTCGATTGC
<i>SMB20625</i>	AGATACAGCGACCACTCTGGA	CAAGGAGCATGGCGAGAT
<i>SMB20820</i>	AAGGCGTGGAGATCATCTGT	ACTGATCCGACCAGAACCAC
<i>SMB21085</i>	CCGTGAGAAAAAGATCGAA	GGCATATGTTGGAATGCTT
<i>SMB21229</i>	CGACGGGAACAACCTATCTGG	ATGACGAGGAAATCGACACC
<i>SMB21356</i>	TTTTCCGAAATAGGCGAAGA	AACCACAGGGAGATCGACAG
<i>SMc00912</i>	TCGGAGCCTATCCTTGACTC	GAACAACCTTGCCGCTTTC
<i>SMc01138</i>	GGCCTGACGAAGTCCTACC	TCACCAAGCAGATCGTTGAG
<i>SMc01312</i>	GTGTGGCGAACTCATGGAC	CAGCAGACGTGATCGTGATG
<i>SMc02823</i>	CGATACGATCACGCTTCTGA	ATGCATAGCGGAAGACCATT

Primer pairs flanking each SNV were designed using Primer3 software (<http://primer3.sourceforge.net/>) and used for PCR amplification. PCR products were then subjected to Sanger sequencing.

Table S3. Primers used in qRT-PCR analysis.

Gene ID	Gene	Forward (5'→3')	Reverse (5'→3')
SMa1227	<i>fixJ</i>	CTCGTGACGGACCTGAGAAT	GCAGCAACCAGATGTTTCAGA
SMa0815	<i>nifA</i>	CCTTGCAAGAGCATTCTTC	TCTTTGACCTGGCGAGAGTT
SMa0825	<i>nifH</i>	TCCACGACCTCCCAAATAC	CGCACTTGATGCCTCTGTAA
SMa1225	<i>fixK1</i>	CATTCTTTCTTTGCCGAAGC	CGCAAAGATCGACGAGAAAT
SMa0762	<i>fixK2</i>	AAGCCAAACCACAGTCCATC	CATCTGAAAGGAGGCGGTAG
SMa1220	<i>fixN1</i>	AATGGTACGTCGACCTCTGG	AGATTGTTGACGACGTGCAG
SMa1216	<i>fixO1</i>	ATCGAGAAGGTCGAGGGAAT	AAAGGGATGGTCGTACATGG
SMa1214	<i>fixQ1</i>	CTCGCAATGACGTTGTTCTT	CCTCCTTTAACGGGATGACA
SMa1213	<i>fixP1</i>	CAGCTTCTATGCCACGATCA	CGGATTTGCGATGATCTCTT
SMa0765	<i>fixN2</i>	GGCATGGTCTTCTCCATCAT	TGTAGTGGCTCAGCGAATTG
SMa0822	<i>fixA</i>	TGGCGAAGATTCTCTCCATT	ACTCCACTTGACGACCTTGG
SMa0834	<i>fdxB</i>	ATCCAGATGGATGCCAAAAT	ATTGAGCTCACCACCGAAAT
SMc00768	<i>gltA</i>	AAGGCCAAAGACAAGAACGA	CGATGTTCCGGTAGAGCTTC
SMc02581	<i>acnA</i>	GATTGCCGACTTCAACCAGT	AACGCCCTCCTCCTTGTACT
SMc01124	<i>icd</i>	AACCTGGACGAATCGATCAC	TTCCTCGTCGAACACCTTCT
SMa0811	<i>sucA</i>	AAGACCGTCGTCCAGCTCTA	CGACCTCCTTCAACTGCTTC
SMc01183	<i>sucD</i>	TGTTCCAGACGACCAATGAA	CCTCGTCTTTCAGGAAGTGC
SMc00768	<i>aceA</i>	GGACGCTATTCCATCTGGTC	CGAGAACGTTGCGATTGTAG
SMc02581	<i>glcB</i>	TTCTTTTCCGCCTTCTCTGA	CGATCTCGGAATCGACATT
SMc00646	<i>rpoH1</i>	GTGAGGAAGAGGTCGTCTCG	TCAGAACCTTCATGGCATTG
SMc01235	<i>uvrA</i>	CTTCCAGCGCGTAAAGGTAG	GCGAGCGTAAGACAGGTTTC
SMc04231	<i>uvrB</i>	CGGTGAAGATCTACGCCAAT	CGAGCATCTCGATGTCGTAA
TC106906	<i>GH3</i>	ACTTAACGGTGCCACTGACC	AACTGACGACGGTCCATTTC

Table S4. Conditions for the collision induced dissociation (CID) ion transmissions of the single amino acids determined by using the Thermo Scientific AutoSRM application run under Chromeleon 7.2 software package.

Aminoacid	Time (min)	Precursor ion <i>m/z</i>	Product ion <i>m/z</i>	Collision energy (eV)	Scan time (sec)
Ala	10	116.1	45	25	0.15
Ala	10	147.1	73.1	15	0.15
Val	13.4	144.1	73	10	0.15
Val	13.4	218.1	73.1	15	0.15
Pro	15.4	142.1	45	30	0.15
Pro	15.4	216.1	147.1	10	0.15
Nor	16.1	158.1	73.1	15	0.15
Nor	16.1	232.1	147.1	10	0.15
Ser	17.2	204.1	188.1	5	0.15
Ser	17.2	204.1	73.1	15	0.15
Thr	17.8	218.1	73.1	15	0.15
Thr	17.8	291.1	101.1	10	0.15
Asp	20.7	232.1	188.1	5	0.15
Asp	20.7	218.1	73.1	15	0.15
Glu	22.5	246.1	128.1	10	0.15
Glu	22.5	230.1	147.1	10	0.15

The conditions reported in the table were determined by using the Thermo Scientific AutoSRM application run under Chromeleon 7.2 software package.

Table S5. Summary of mutations detected as unique to the 1021 bacteroids.

Gene ID ^a	Position	Type	bp Insertion/Deletion	Codon change ^b	Aminoacid change
<i>SMa0056</i>	pSymA:28572-28573	frameshift	A/AG	-	-
<i>SMa0056</i>	pSymA:28580-28581	frameshift	T/TG	-	-
<i>SMa0056</i>	pSymA: 28572-28573	frameshift	G	-	-
<i>SMa0171</i>	pSymA:93633	frameshift	CG/G	-	-
<i>SMa0461</i>	pSymA:245439	missense	-	tcN/tcG	-
<i>SMa0461</i>	pSymA:245660	missense	-	gTt/gAt	V/D
<i>SMa0673</i>	pSymA:359192	missense	-	gGt/gAt	G/D
<i>SMa1132</i>	pSymA:618529	frameshift	GC/C	-	-
<i>SMa1473</i>	pSymA:810470-810472	frameshift	C/CGT	-	-
<i>SMa1608</i>	pSymA:897582	missense	-	cTg/cGg	L/R
<i>SMa2043</i>	pSymA:1156230-1156232	frameshift	CGGG/CGG	-	-
<i>SMa2043</i>	pSymA:1156437	frameshift	CG/G	-	-
<i>SMa2193</i>	pSymA:1228869	frameshift	TA/A	-	-
<i>SMa2307</i>	pSymA:1287071	frameshift	CA/A	-	-
<i>SMa2307</i>	pSymA:1287083	frameshift	CA/A	-	-
<i>SMa2307</i>	pSymA:1286991-1286992	frameshift	T/CATC	-	-
<i>SMa5034</i>	pSymA:1339737	frameshift	AC/C	-	-
<i>SMa5034</i>	pSymA:1339763	frameshift	CA/A	-	-
<i>SMb20332</i>	pSymB:335974	frameshift	GT/T	-	-
<i>SMb20332</i>	pSymB:335974	frameshift	-	-	-
<i>SMb20558</i>	pSymB:583800-583801	frameshift	A/AG	-	-
<i>SMb20568</i>	pSymB:1581711-1581712	frameshift	C/CG	-	-
<i>SMb20596</i>	pSymB:1609669-1609670	Frameshift	A/AC	-	-
<i>SMb20625</i>	pSymB:1646596	frameshift	AC/C	-	-
<i>SMb20625</i>	pSymB:1646600-1646601	frameshift	C/CG	-	-
<i>SMb20654</i>	pSymB:1455987-1455988	frameshift	G/GC	-	-
<i>SMb20664</i>	pSymB:1465448-1465449	frameshift	G/GC	-	-
<i>SMb20688</i>	pSymB:1491989-1491990	frameshift	G/GC	-	-
<i>SMb20688</i>	pSymB:1492005	frameshift	GA/A	-	-
<i>SMb20816</i>	pSymB:604526-604527	frameshift	C/CT	-	-
<i>SMb20820</i>	pSymB:609534	missense	-	Cat/Aat	H/N
<i>SMb20841</i>	pSymB:632007-632008	frameshift	G/GC	-	-
<i>SMb20857</i>	pSymB:1262639-1262640	frameshift	A/AG	-	-
<i>SMb20857</i>	pSymB:1262639-1262640	frameshift	G	-	-
<i>SMb21022</i>	pSymB:646477-646478	frameshift	G/GC	-	-
<i>SMb21055</i>	pSymB:688822-688823	Frameshift	G/GC	-	-
<i>SMb21058</i>	pSymB:692914-692915	Frameshift	A/AC	-	-
<i>SMb21063</i>	pSymB:698625-698626	Frameshift	A/AC	-	-
<i>SMb21089</i>	pSymB:732199-732200	frameshift	G/GC	-	-
<i>SMb21089</i>	pSymB: 732199-732200	frameshift	C	-	-
<i>SMb21098</i>	pSymB:741275-741276	Frameshift	A/AG	-	-
<i>SMb21225</i>	pSymB:789801-789802	missense	-	cCG/cGC	P/R
<i>SMb21243</i>	pSymB:811242-811243	missense	-	caCGtt/caGCtt	HV/QL
<i>SMb21257</i>	pSymB:829338-829339	frameshift	A/AG	-	-
<i>SMb21432</i>	pSymB:1362283-1362284	frameshift	A/AG	-	-
<i>SMb21463</i>	pSymB:1390093-1390094	frameshift	G/GC	-	-
<i>SMb21490</i>	pSymB:1413156	frameshift	GC/C	-	-
<i>SMb21496</i>	pSymB:1417679	frameshift	CG/G	-	-
<i>SMb21503</i>	pSymB:1428080-1428081	frameshift	G/GC	-	-
<i>SMb21541</i>	pSymB:1071681-1071682	frameshift	G/GC	-	-
<i>SMb21541</i>	pSymB:1071681	missense	V/L	Gtg/Ctg	-
<i>SMb21707</i>	pSymB:1614944	frameshift	AT/T	-	-
<i>SMb21707</i>	pSymB:1614982-1614983	frameshift	G/GC	-	-
<i>SMc00177</i>	Chr:1986166	missense	-	cAg/cCg	Q/P
<i>SMc00858</i>	Chr:929558	missense	-	gCg/gGg	A/G

<i>SMc01312</i>	Chr:1477181	missense	-	gCg/gAg	A/E
<i>SMc02349</i>	Chr:2771499	missense	-	Tgc/Ggc	C/G
<i>SMc02757</i>	Chr:43233	missense	-	Gcg/Ccg	A/P
<i>SMc02793</i>	Chr:799	missense	-	Tcg/Ccg	S/P
<i>SMc02794</i>	Chr:3654130-3654135	frameshift	GTCGCAT/GTCGCA TCGCAT	-	-
<i>SMc02983</i>	Chr:3104144	missense	-	gCg/gGg	A/G
<i>SMc03989</i>	Chr:2987197-2987198	frameshift	G/GC	-	-
<i>SMc04028</i>	Chr:3029642	missense	-	cGc/cCc	R/P
<i>SMc04272</i>	Chr:2202002	missense	-	gCg/gGg	A/G
<i>SMc04357</i>	Chr:2237190-2237193	missense	-	gGTGGc/gACA Ac	GG/DN
<i>SMc04357</i>	Chr:2237194-2237195	missense	-	ggCGgc/ggCAA CGgc	GG/GNG

^aGenes highlighted in grey were selected for PCR and Sanger sequence analyses. ^bUpper case letters represent mutated bases.

Table S6. Summary of mutations detected as unique to the RD64 bacteroids.

Gene ID [§]	Position	Type	bp Insertion/Deletion	Codon change [¶]	Aminoacidchange
<i>SMA0233</i>	pSymA: 130316	missense		Ccc/Gcc	P/A
<i>SMA0745</i>	pSymA:401010	missense		Gtc/Atc	V/I
<i>SMA0785</i>	pSymA:428086	missense		Ggc/Tgc	G/C
<i>SMA0937</i>	pSymA:520887-520890	frameshift		A/AAAAAA	
<i>SMA1315</i>	pSymA:718902	missense		Cgc/Ggc	R/G
<i>SMA1539</i>	pSymA: 850708	missense		gCg/gGg	A/G
<i>SMA2061</i>	pSymA: 1162842	missense		Acc/Ccc	T/P
<i>SMB20683</i>	pSymB: 1485681	missense		Gcg/Ccg	A/P
<i>SMB21085</i>	pSymB:723806	missense		gTc/gCc	V/A
<i>SMB21085</i>	pSymB:724102-724103	missense		gtc/gATCAGTtc	V/DQF
<i>SMB21229</i>	pSymB: 793473	missense		gGc/gTc	G/V
<i>SMB21300</i>	pSymB: 881781	missense		cGc/cCc	R/P
<i>SMB21356</i>	pSymB:1022521	missense		aaG/aaT	K/N
<i>SMB21476</i>	pSymB:1400872	frameshift	CC/CCATCCAGC	-	-
<i>SMB21506</i>	pSymB: 1432384	missense		Gcg/Tcg	A/S
<i>SMB21671</i>	pSymB: 1070087-1070088	frameshift	CTTGTGGG	-	-
<i>SMc00722</i>	Chr:2860009	missense		gaG/gaC	E/D
<i>SMc00912</i>	Chr:871102	missense		Gtc/Atc	V/I
<i>SMc01053</i>	Chr:1583930	missense		gTc/gGc	V/G
<i>SMc01138</i>	Chr:413477	missense		cGc/cAc	R/H
<i>SMc01394</i>	Chr: 458847	missense		Gat/Tat	D/Y
<i>SMc01468</i>	Chr: 2315665	missense		tGt/tTt	C/F
<i>SMc01554</i>	Chr:2495818-2495819	missense		gtc/gCAAtc	V/AI
<i>SMc02300</i>	Chr: 673665	missense		Ggc/Tgc	G/C
<i>SMc02303</i>	Chr:676836	missense		cCc/cTc	P/L
<i>SMc02823</i>	Chr:170764	missense		Cgc/Agc	R/S
<i>SMc03257</i>	Chr:3373110	missense		cGc/cTc	R/L

[§] Genes highlighted in grey were selected for PCR and Sanger sequence analyses. [¶]Upper case letters represent mutated bases.