# Supporting Information

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### SI Methods

**Mutant Construction.** ***RU1979.*** Primers (AA*ACTAGT*CATAGACCGAATCGACCAC; AA*ACTAGT*GGGTCTTTTCAAAGGATA) were used to amplify the Rlv3841 *aapJQMP* operon flanked by SpeI sites (indicated in italics in the primer sequence). A 3.6-kb SpeI/XhoI fragment carrying the *aapJ* gene with flanking DNA was cloned into pJQ200SK (1). A 600-bp SalI fragment was deleted from *aapJ*, causing an in-frame deletion. This plasmid pRU1228 was used to replace the *aapJ*::Tn5*lacZ* marker in RU543 (2) by using *sacB* mutagenesis, resulting in strain RU1978. The deletion was PCR-verified by using primers (AA*ACTAGT*CATAGACCGAATCGACCAC; GATGAACAGCCAGTTGACGAGATG). The in-frame nature of the deletion was confirmed by sequencing this PCR product.

A 2.5-kb fragment containing *braC* and flanking DNA was amplified by using primers (TTTTT*GTCGAC*CCGCCCTCAGGCTTTCTCAC; TTTTT*GTCGAC*ATCGTCGCCCGCCATATCGC). This PCR product was cloned as a SalI fragment (italics in the primers) into pJQ200SK, and a SmaI-flanked ΩSpec resistance cassette (3) was inserted into a 181-bp XmnI deletion within *braC*. The resulting plasmid, pRU1255, was then used to select a Spec-resistant and gentamicin (Gm)-sensitive homogenote in RU1978 by using *sacB* mutagenesis, resulting in strain RU1979. The mutant was PCR-verified by using the above primers, which amplified only a fragment expected from the ΩSpec insertion and no shorter wild-type fragment.

***RU2267.*** A pJQ200SK derivative, pRU1137 (4), containing the *braDEFG* operon with a 153-bp EcoRV deletion in *braEF*, into which an ΩTet resistance cassette (3) has been cloned, was used to select a Tet-resistant and Gm-sensitive homogenote in RU1978 by using *sacB* mutagenesis. The resulting strain was named RU2267 and was PCR-verified by using primers (GCTTCGTCTCGCCGGAATCCTT; ATAGGGGCCGACACCGATGA), producing only a fragment expected from the mutant.

***RU2281.*** A 3.5-kb fragment containing *braC3* and flanking DNA was amplified by using primers (CACC*TCTAGA*CCGCTGATCATCGAGAAGCAC; *ACTAGT*GTCGTGCATTCGTGCTCGGT) and cloned as an XbaI/SpeI fragment (italics in the primers) into pJQ200SK. The Invitrogen GeneJumper ($K_m^r$) kit was then used to carry out in vitro mutagenesis. A $K_m$-resistant clone was isolated with the GeneJumper Transposon insertion at base pair 361 of the *braC*3 ORF. The resulting plasmid, pRU1536, was then used to select a $K_m$-resistant and Gm-sensitive homogenote in RU1979 by using *sacB* mutagenesis, resulting in strain RU2281.

***LMB65.*** A 2.6-kb fragment containing *ilvD* and flanking DNA was amplified by using primers (*ACTAGT*TGAAATATAGCCCTTAAACG; *ACTAGT*TCGATTGCGATGCGAAAGAA) and cloned as a SpeI fragment (italics in the primers) into pJQ200SK, and a SmaI-flanked ΩSpec resistance cassette (3) was inserted into an 84-bp EcoRV deletion within *ilvD*. The resulting plasmid, pRU2202, was then used to select a Spec-resistant and Gm-sensitive homogenote in Rlv3841 by using *sacB* mutagenesis, resulting in strain LMB65. The mutant was PCR-verified by using primers (GATGACGCGATCTTCGGTGG; CGGTTTACAAGCATAAAGC).

***LMB66 and LMB69.*** A 2.6-kb fragment containing *leuD* and flanking DNA was amplified by using primers (*ACTAGT*GAAAGCCGAAGCAAGCAGCC; *ACTAGT*TCGCGCCTCTCCACCTCGAC) and cloned as an SpeI fragment (italics in the primers) into pJQ200SK, and a SmaI-flanked ΩSpec resistance cassette (3) was inserted into a 54-bp NruI/PsiI deletion within *leuD*. The resulting plasmid, pRU2203, was then used to select a Spec-resistant and Gm-sensitive homogenote in Rlv3841 and RU2267 by using *sacB* mutagenesis, resulting in strains LMB66 and LMB69, respectively. The mutants were PCR-verified by using primers (TCGGCTCATGGCGAATTAGT; CGGTTTACAAGCATAAAGC).

**Complementing Plasmids.** **pJP2 Pa*BraCDEFG.*** An overlap PCR strategy was used to clone the Rlv3841 *aapJ* promoter region to a fragment of *P. aeruginosa braC*. Primers (CACCCGCGCGTCCCGTAAGTGTAT; CGCTGAGTACCCTTCTTCATTTTCCCAACCTTTTCCGTTG) were used to amplify the Rlv3841 *aapJ* promoter region (*aapJ*$_p$) from a plasmid carrying the entire *aapJQMP* operon. Primers (CAACGGAAAAGGTTGGGAAAATGAAGAAGGGTACTCAGCG; TGTAGCGTTCGGCGATGAACTTGCC) were used to amplify the *Pa braC* from a plasmid carrying the entire *Pa braCEDFG* operon. A third PCR was performed by using primers (CACCCGCGCGTCCCGTAAGTGTAT; TGTAGCGTTCGGCGATGAACTTGCC) and the 2 initial PCR products as template. PCRs were carried out by using *Pfu* Turbo (Bioline) at 58 °C annealing. The resulting overlap PCR product was cloned into pCR-BluntII-TOPO (Invitrogen). The entire *Pa braCEDFG* operon was cloned into pJP2 (5) as a 6-kb SacI/XbaI fragment. This plasmid was then HindIII/AgeI-digested to remove the original promoter and start site of *Pa braC* and was replaced by a HindIII/AgeI fragment containing the *Rl aapJ*$_p$ *Pa braC* overlap PCR product from pCR-BluntII-TOPO, resulting in plasmid pJP2 *Pa*BraCDEFG.

**pJP2 Ec*LivKHMGF.*** Primers (<u>CAACGGAAAAGGTTGGGAAA</u>ATGAAACGGAATGCGAAAAC; TTATTACCCGCCTAAATACGTCTAGA) were used to amplify the entire *livKHMGF* operon from *E. coli* DH5α total DNA. The PCR was performed by using the high-fidelity Phusion enzyme (Finnzymes) at 66 °C annealing, and the product was cloned into pJET/blunt (Fermentas). The first primer contained the 20 bp upstream of the Rlv3841 *aapJ* start codon (underlined) to ensure translation in Rlv3841. From a clone with the right orientation, a 4.9-kb XbaI/EcoRI fragment was cloned into pJP2neo digested with XbaI/MunI, resulting in pJP2 *Ec*LivKHMGF. (pJP2neo is a pJP2 derivative containing a 1,330-bp fragment spanning the entire Tn5 nptII, including the promoter cloned into the filled-in KpnI side of the pJP2 MCS, driving constitutive expression into the GUS reporter gene.)

**pJP2 Pf*GltIJKL.*** Primers (AATT*GGATCC*GCGCGTCCCGTAAGTGTAT; GAATATGGGGAACGATGCGCATTTTCCCAACCTTTTCCGTTG) were used to amplify the Rlv3841 *aapJ*$_p$. Primers (ATGCGCATCGTTCCCCATATTC; TTAAT*TCTAGA*GCACTGCCTGTATTCACCGCAC) were used to amplify the entire *P. fuorescens* SWB25 *gltIJKL* operon from genomic DNA. Primers (AATT*GGATCC*GCGCGTCCCGTAAGTGTAT; TTAAT*TCTAGA*GCACTGCCTGTATTCACCGCAC) were used to amplify the overlap PCR product by using the previous PCR products as template. All PCRs were performed by using the high-fidelity Phusion enzyme at 66 °C annealing. The final PCR product was cloned into pJET/blunt, and a 3.8-kb BamHI/XbaI (italics in the primers) was cloned into a BamHI/XbaI-digested pJP2, resulting in pJP2 *Pf*GltIJKL.

**pJP2 nifH$_p$*GdhA.*** Primers (ATGGATCAGACATATTCTCT; TTAAATCACACCCTGCGCCA) were used to amplify the *gdhA* gene from *E. coli* DH5α total DNA. Primers (ACTGATTGCCCGACTATGCA; AGAGAATATGTCTGATCCATGTTT-

GGCGTTCCTTCATGTG) were used to amplify the Rlv3841 $nifH_p$. Primers (TTAAATCACACCCTGCGCCA; ACTGATTGCCCGACTATGCA) were used to amplify the overlap PCR product by using the previous PCR products as template. All PCRs were performed by using the high-fidelity Phusion enzyme at 66 °C annealing. The final PCR product was cloned into pJET/blunt, and from a clone with the right orientation a 2.4-kb BglII/XbaI was cloned into a BamHI/XbaI-digested pJP2, resulting in pJP2 $nif_p$GdhA.

***Primers.*** qRT-PCR Primers were *ilvC* (GCCAAGAAGGTCGCCGTCATC; AACCGGCCTTGAGTGCGATG), *ilvE1* (CCGTCGATACATCACCCCGTTC; CGTCGAGCCCAGCCACATG), *ilvE2* (GCACTGGAGACACCCGGCTTC; CGAGCTCCAGAGGACGCCTTG), *leuB* (CTTACGAAGTGCGCCCGGAAG; CGCAGCAAGGGCCGGATAG), *leuC* (AAGTGGCGCGCGCTTGACTATA; ATCCTCGATGCGGCCGTTG), and *leuD* (CTCTTCGCGGAAGCCCGTTATA; GGAGCCGCAGCCGAAATTG).


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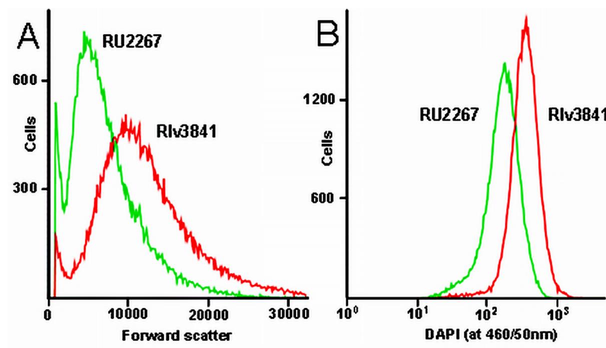


**Fig. S1.** Flow cytometry analysis of Rlv3841 and RU2267 bacteroids. (*A*) Forward scatter as an indication of size. (*B*) DAPI intensity at 460 nm as a measure of DNA content. Bacteroids of Rlv3841 and RU2267 had chromosome numbers of ≈8 and ≈5, respectively, relative to cultured bacteria. In each experiment 50,000 cells were analyzed.

**Table S1. Microarray and qRT-PCR results from 21-day-old nodule bacteroids**

| Gene | Array* | *P* | qRT-PCR* | *P* |
|---|---|---|---|---|
| RL1953 *ilvA* | 0.447 | 0.150 | ND | ND |
| RL3338 *ilvG* | 0.458 | 0.143 | ND | ND |
| RL3244 *ilvH* | 0.304 | 0.080 | ND | ND |
| RL3245 *ilvI* | 0.497 | 0.106 | ND | ND |
| RL3205 *ilvC* | 0.097 | 0.003 | 0.109 | 0.013 |
| RL1326 *ilvE1* | 0.439 | 0.107 | 0.235 | 0.065 |
| RL3200 *ilvE2* | 0.351 | 0.000 | 0.145 | 0.051 |
| RL1538 *leuA1* | 0.532 | 0.068 | ND | ND |
| RL4707 *leuB* | 0.241 | 0.023 | 0.087 | 0.018 |
| RL4555 *leuC* | 0.276 | 0.020 | 0.067 | 0.002 |
| RL4705 *leuD* | 0.178 | 0.002 | 0.068 | 0.012 |

ND, not determined.

*Expression relative to AMS succinate/$NH_4Cl$ cultures for expression of genes of the branched-chain amino acid biosynthetic pathways. Number of replicates $n = 3$.