SUPPLEMENTARY MATERIAL

Biochemical and genetic basis of indole-3-acetic acid (auxin phytohormone) degradation by the plant growth promoting rhizobacterium *Paraburkholderia phytofirmans* PsJN.

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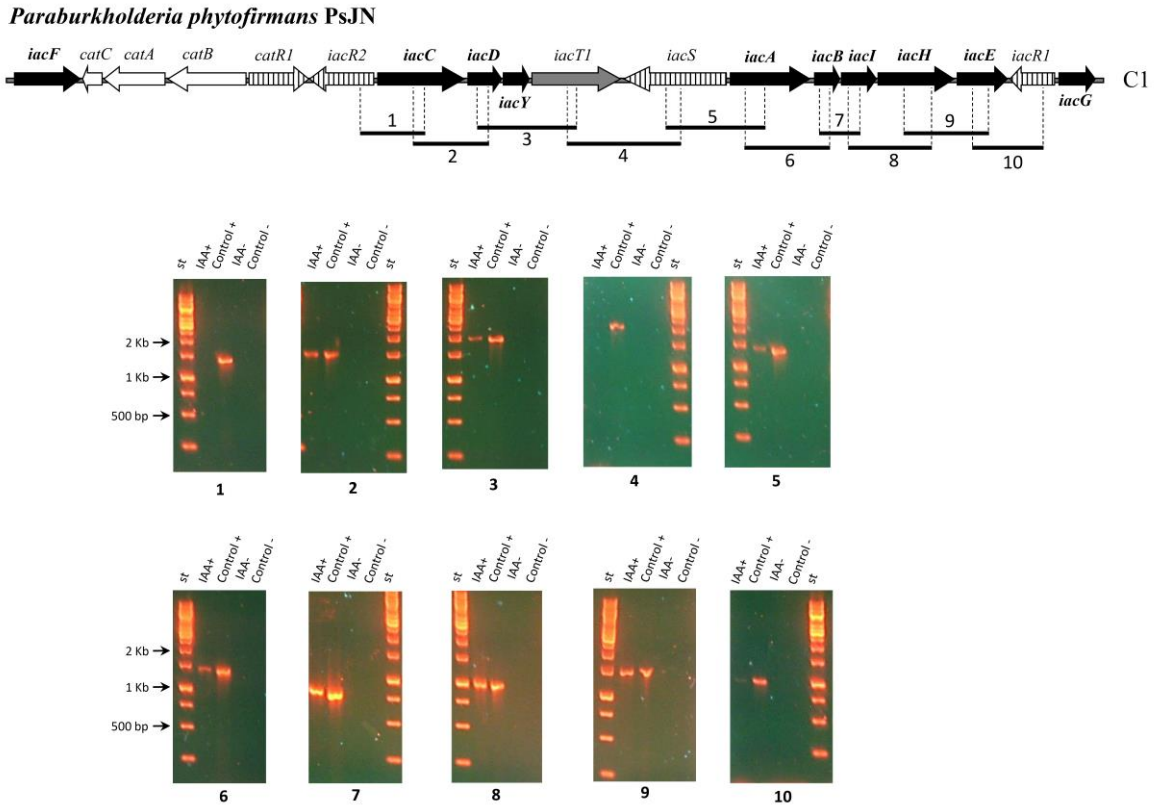


Figure S1. Transcriptional analysis of *iac* genes apparently grouped in operon functional units. The numbers correspond to the DNA fragments amplified by PCR using primer pairs shown in Table 1. 1 (1.37 kb): Mut iacR2Rv-Mut iacCRv; 2 (1.46 kb): Mut iacCFw-Mut iacDRv; 3 (1.90 kb): Mut iacDFw-Mut iacT1Rv; 4 (1.91 kb): Mut iacT1Fw-Mut iacSFw; 5 (1.68 kb): Mut iacSRv-Mut iacARv; 6 (1.35 kb): Mut iacAFw-Mut iacBRv; 7 (0.78 kb): Mut iacBFw-Mut iacIRv; 8 (1.01 kb): Mut iacIFw-Mut iacHRv; 9 (1.41 kb): Mut iacHFw-Mut iacERv; 10 (0.97 kb): Mut iacEFw-Mut iacR1Rv. IAA+, cDNA of strain PsJN grown in IAA; Control +, strain PsJN genomic DNA; IAA-, no reverse transcriptase control of IAA+; Control -, no DNA control.

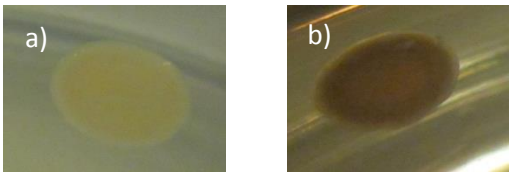


Figure S2. *Paraburkholderia phytofirmans* PsJN *catA* gene mutant cells turned brown a medium supplemented with indole-3-acetic acid (IAA). A) Wild type strain and B) *catA* mutant grown in fructose plates plus 1 mM IAA.

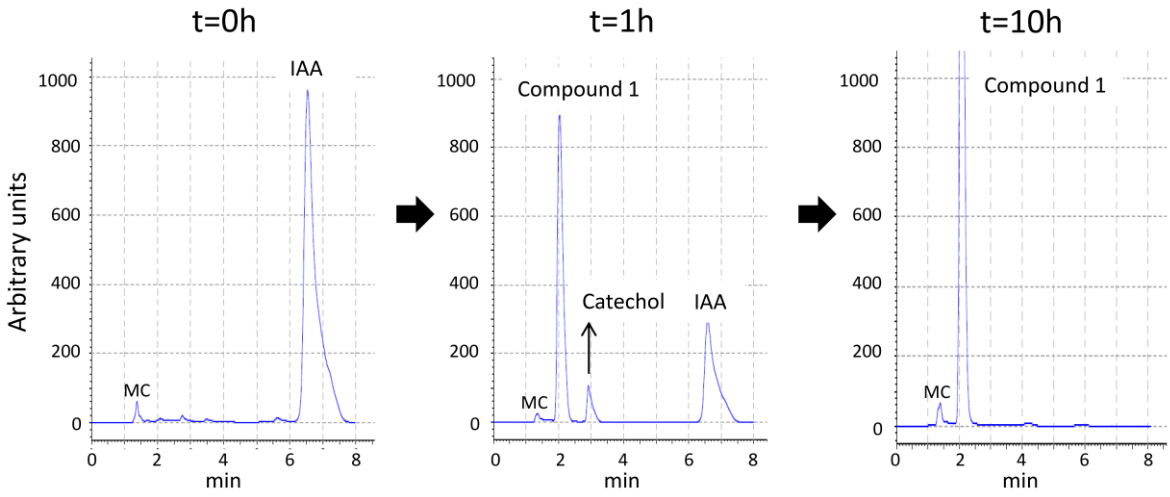


Figure S3. High-performance liquid chromatography ultraviolet chromatograms of supernatants from cultures of *Cupriavidus pinatubonensis* JMP134 derivatives harboring *iacABIHECDGF* genes (strain JMP134*iac*1). This strain was exposed to 1 mM indole-3-acetic acid (IAA) for 10 h. Catechol appears only transiently at 1 h due to this strain is able to metabolize it. Retention times for IAA, catechol and compound 1, were 6.75, 3.05 and 2.04 min, respectively. MC, a signal found in controls without bacteria.

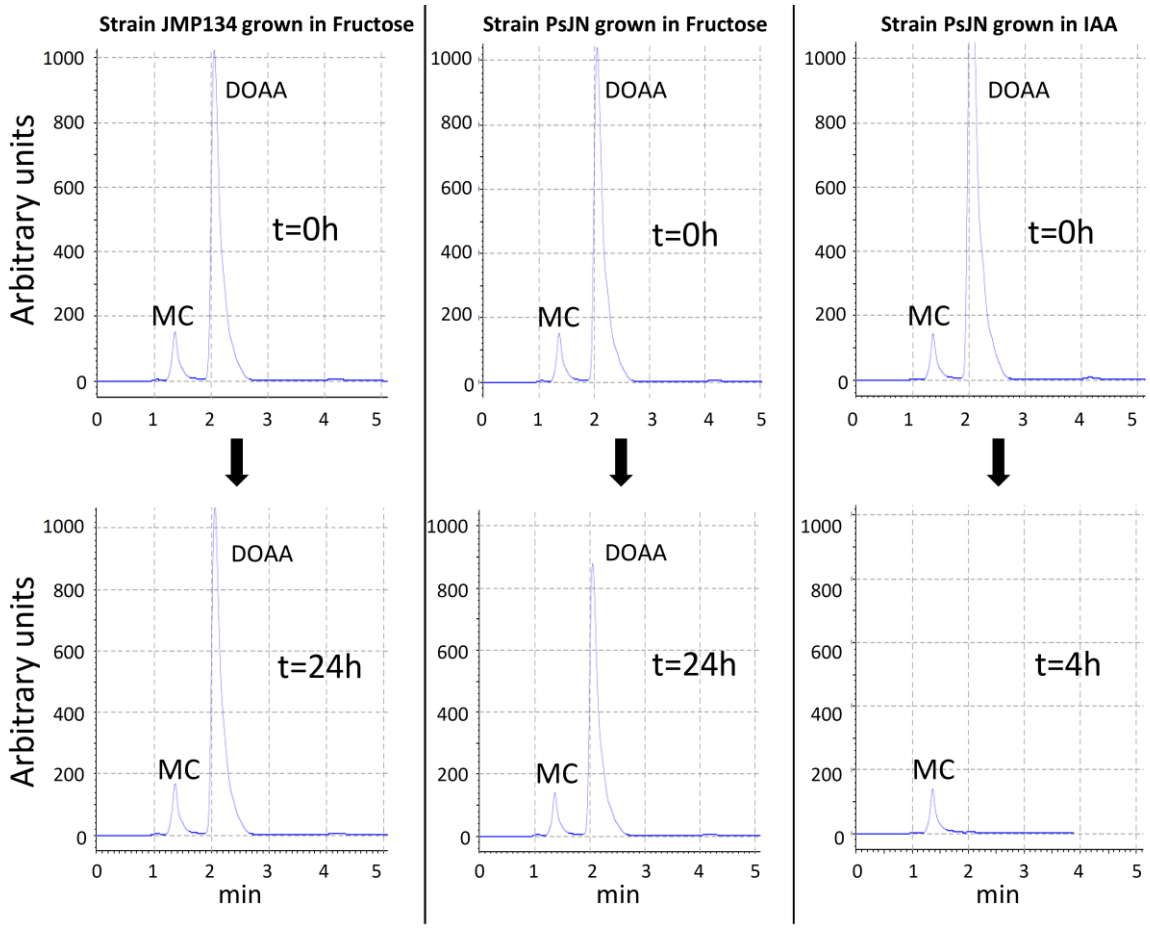


Figure S4. High-performance liquid chromatography ultraviolet chromatograms of supernatants from cultures of *Cupriavidus pinatubonensis* JMP134 and *Paraburkholderia phytofirmans* PsJN grown in fructose or IAA to stationary phase, washed and later exposed to supernatant containing dioxindole-3-acetic acid (DOAA). Retention time for compound 1 was 2.04 min. MC, a signal found in controls without bacteria.

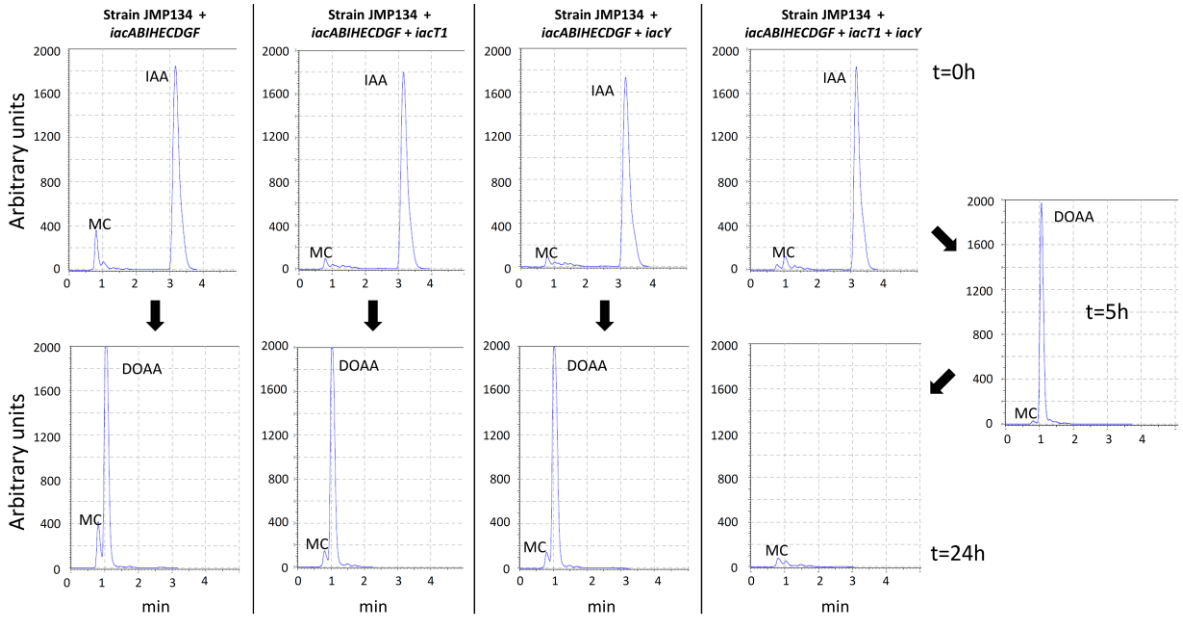


Figure S5. High-performance liquid chromatography ultraviolet chromatograms of supernatants from cultures of *Cupriavidus pinatubonensis* JMP134 derivatives harboring *iacABIHECDGF* genes plus *iacT1* and/or *iacY* genes, after 0 h and 24 h of exposure to 1 mM indole-3-acetic acid (IAA). Retention times for IAA and dioxindole-3-acetic acid (DOAA) were 3.18 and 1.03 min, respectively. MC, a signal found in controls without bacteria.

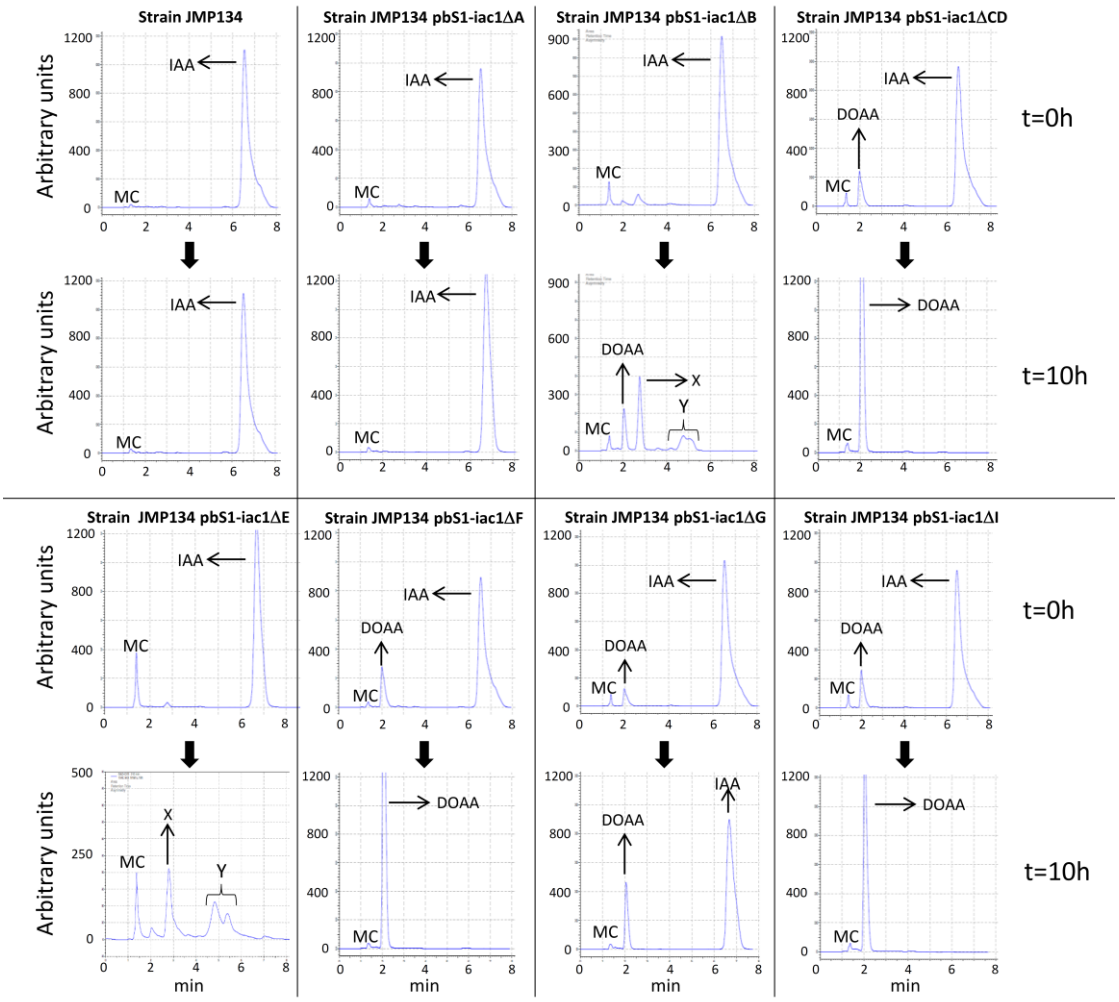


Figure S6. High-performance liquid chromatography ultraviolet chromatograms of supernatants from cultures of *Cupriavidus pinatubonensis* JMP134 harboring pbS1-iac1ΔA, pbS1-iac1ΔB, pbS1-iac1ΔCD, pbS1-iac1ΔE, pbS1-iac1ΔF, pbS1-iac1ΔG and pbS1-iac1ΔI after 0 h and 10 h of exposure to 1 mM indole-3-acetic acid (IAA). Retention times for IAA, dioxindole-3-acetic acid (DOAA), compound X and compound Y were 6.75, 2.04, 2.78 and 4.85-5.30 min, respectively. Despite the very short period between the initial time of IAA exposure and the zero time sampling (usually less than 1-2 min), in some strains/conditions, the DOAA signal at time 0 was detected. MC, a signal found in controls without bacteria.

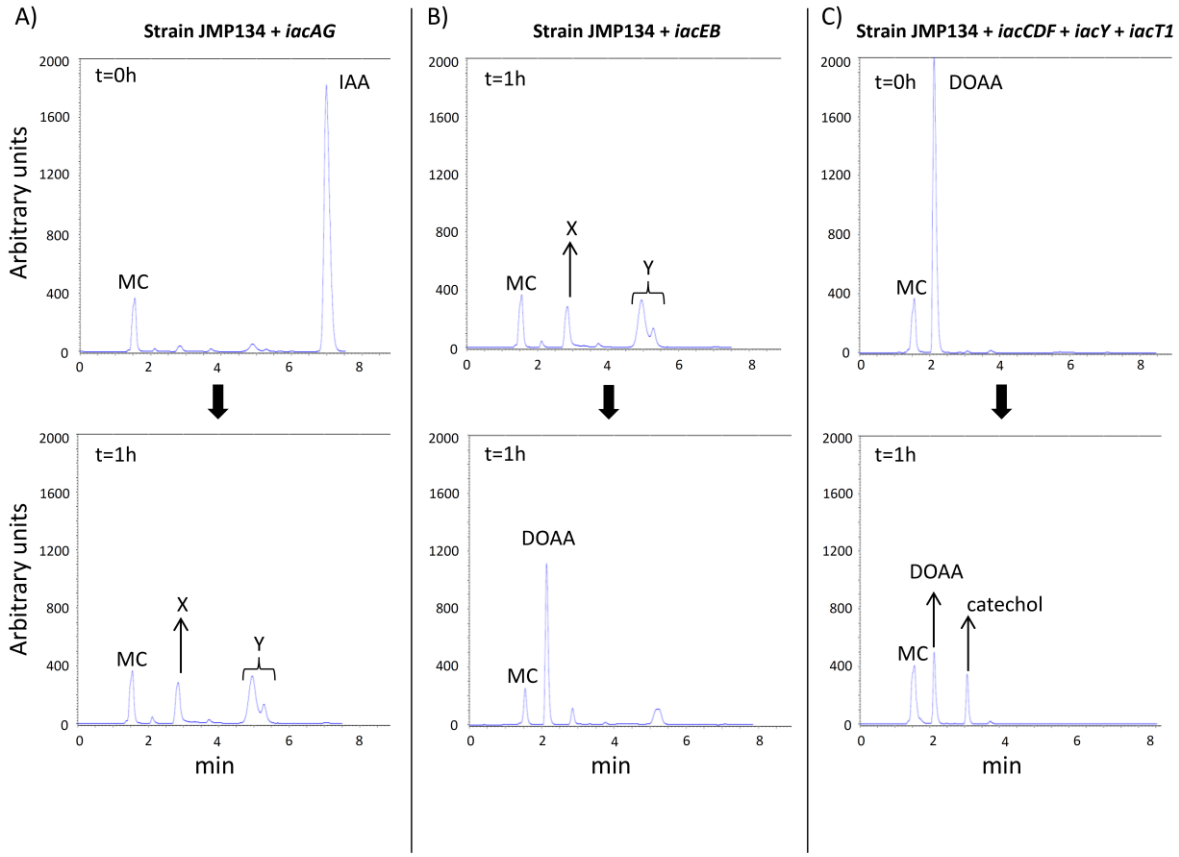


Figure S7. High-performance liquid chromatography ultraviolet chromatograms of supernatants from cultures of *Cupriavidus pinatubonensis* JMP134 derivatives harboring A) *iacAG*, B) *iacEB* and C) *iacCDFT1Y* genes, after 0 h and 1 h of exposure to 1 mM indole-3-acetic acid (IAA), or supernatants containing compounds designated X-Y, or dioxindole-3-acetic acid (DOAA). Retention times for IAA, DOAA, compound X and compound Y were 6.75, 2.04, 2.78 and 4.85-5.30 min, respectively. MC, a signal found in controls without bacteria.

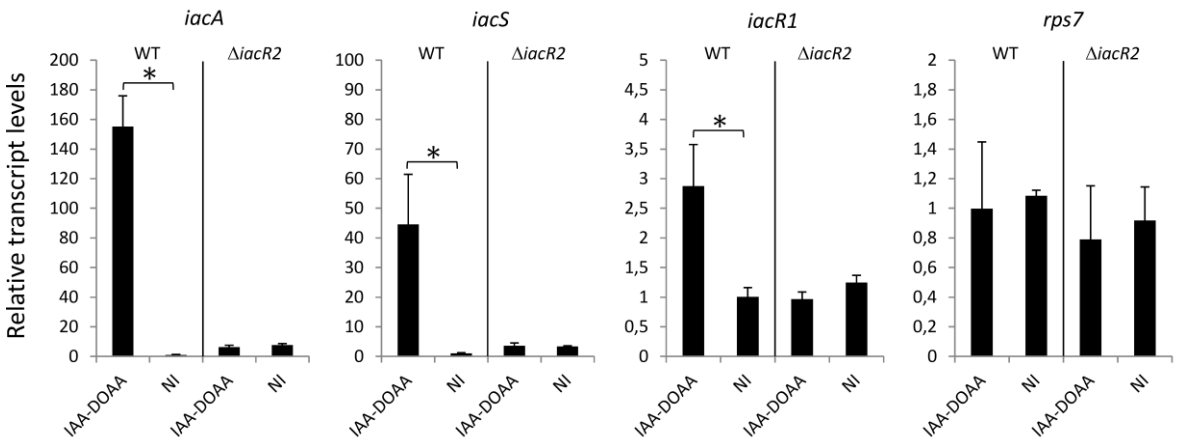


Figure S8. IacR2 regulator positively controls transcription levels of *iacS* and *iacR1* gene encoded regulators. Transcript levels of *iacA*, *iacS* and *iacR1* from cells of strain PsJN wild type (WT) and *iacR2* mutant derivative (Δ*iacR2*) grown in fructose (mid-log phase) exposed 1 h to mixtures of indole-3-acetic acid (IAA) and dioxindole-3-acetic acid (DOAA). It should be noted that transcript levels of the ribosomal protein S7 (*rpS7*) gene, used as expression controls, remained unchanged under these conditions. Transcript levels were normalized to the average value of the transcript levels in the non-induced (NI) condition. 16S rRNA was used as a reference gene (internal control). All experiments were performed in three biological replicates. Error bars represent standard deviations. Asterisks indicates significant differences between treatments (Student's t-test, $p < 0.05$).

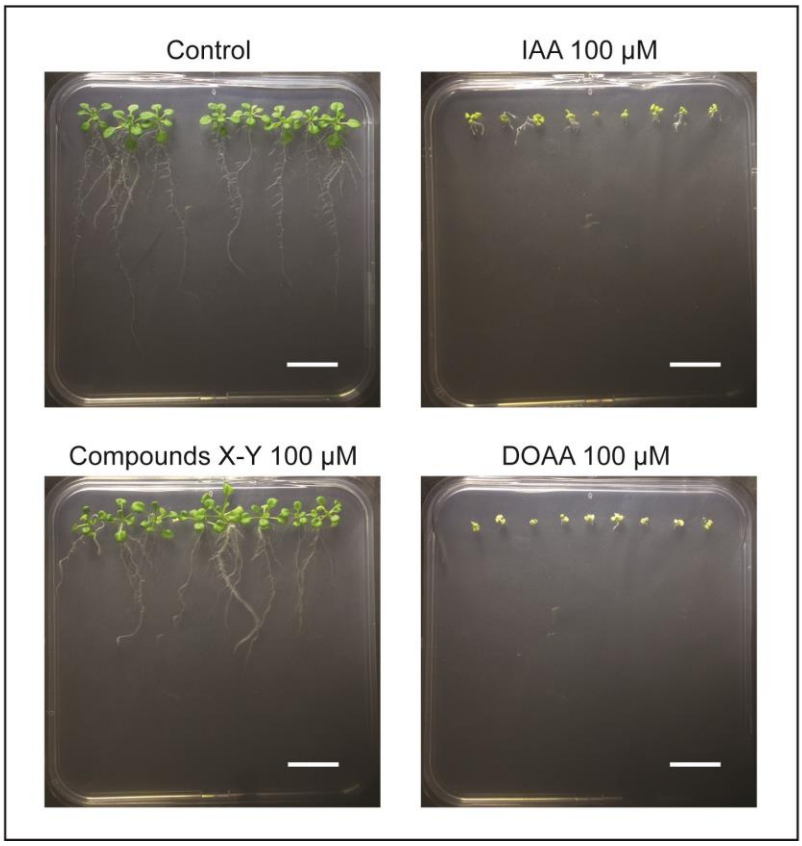


Figure S9. Effects of metabolites of indole-3-acetic acid biodegradation on *Arabidopsis thaliana* plants. Phenotype of *A. thaliana* plants cultivated with indole-3-acetic acid (IAA), dioxindole-3-acetic acid (DOAA), or compounds X-Y. Col-0 *A. thaliana* seeds, previously sterilized, were sown on square Petri dishes with half strength Murashige and Skoog medium (1) 0.8% agar (50% MS) (control); 50% MS supplemented with IAA (100 µM); 50% MS supplemented with DOAA (100 µM) or 50% MS supplemented with compounds X-Y (100 µM). Plates were placed vertically in a growth chamber at 22°C with a photoperiod of 16 h of light and 8 h of dark for 19 days. The assay was done in triplicate (n = 27 plants per treatment). White bars in photographs correspond to 2 cm. Compounds X-Y or DOAA were prepared using 10-fold diluted supernatants of strain JMP134-*iacAG* or JMP134*iac*1 resting cells previously exposed to 1 mM IAA, as the only carbon source for 2 h, respectively.

Table S1. Amino acid identity percentages between *iac* genes encoded products in *Pseudomonas putida* 1290 and *Paraburkholderia phytofirmans* PsJN.

***P. putida* 1290 gene; accession number; Automatic annotation[A]**	***P. phytofirmans* PsJN locus tag**	**Amino acid identity**
iacA; ABY62757; Acyl-CoA dehydrogenase-like	Bphyt_2161, Bphyt_6111	56%, 49%
iacB; ABY62758; Conserved hypothetical protein	Bphyt_2162	57%
iacC; ABY62759; Aromatic ring hydroxylating dioxygenase	Bphyt_2156	62%
iacD; ABY62760; Aromatic ring hydroxylating dioxygenase	Bphyt_2157	44%
iacE; ABY62761; Short-chain dehydrogenase/reductase	Bphyt_2165	52%
iacF; ABY62762; Ferredoxin	Bphyt_2150, Bphyt_4243	41%, 38%
iacG; ABY62763; Flavin reductase	Bphyt_5028, Bphyt_2167, Bphyt_6112	53%, 50%, 47%
iacH; ABY62765; Glu-tRNA amidotransferase	Bphyt_2164	52%
iacI; ABY62766; Conserved hypothetical protein	Bphyt_2163	44%
iacR; ABY62764; Transcriptional regulator MarR family	absent	NA

[A]Automatic annotation: *iac* genes of *P. putida* 1290 (2) have highest BLASTP similarities to sequenced strain *P. putida* GB-1, which possess *iac* genes automatically annotated. NA: not apply.

Table S2. Amino acid identity percentages between additional *iac* genes of *Paraburkholderia phytofirmans* PsJN and proteins with demonstrated genetical and/or biochemical functions.

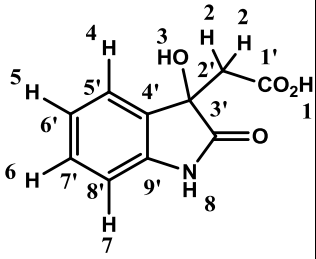
Locus; gene name	Lenght (aa)	Homology with	Identity (%)	Lenght (aa)	Strain	Reference
Bphyt_2158; *iacY*	127	2-hydroxymuconate tautomerase	45	63	*Pseudomonas stutzeri*	3
Bphyt_2159; *iacT1*	438	Shikimate transporter	40	438	*Escherichia coli*	4
Bphyt_6110; *iacT2*	452	Shikimate transporter	41	438	*Escherichia coli*	4
Bphyt_2160; *iacS*	506	Sensor protein FixL	31	505	*Bradyrhizobium japonicum*	5
Bphyt_2166; *iacR1*	220	Response regulator protein TodT	45	227	*Pseudomonas putida*	6
Bphyt_2155; *iacR2*	316	Transcriptional activator NagR	39	301	*Ralstonia sp.* strain U2	7
Bphyt_2152; *catA*	302	Catechol 1,2-dioxygenase 2	98	275	*Acinetobacter lwoffii*	8
Bphyt_1590; *catA2*	311	Catechol 1,2-dioxygenase 1	89	311	*Acinetobacter lwoffii*	8
Bphyt_2153; *catB*	385	Muconate cycloisomerase 1-2	91	385	*Acinetobacter lwoffii*	8
Bphyt_1588; *catB2*	375	Muconate cycloisomerase 1	62	375	*Pseudomonas putida*	9
Bphyt_2151; *catC*	96	Muconolactone delta-isomerase 2	95	96	*Acinetobacter lwoffii*	8
Bphyt_1587; *catC2*	100	Muconolactone delta-isomerase	81	92	*Cupriavidus pinatubonensis*	10
Bphyt_1591; *benA*	452	Benzoate dioxygenase large subunit	60	461	*Acinetobacter calcoaceticus*	11

Table S3. Growth of *Cupriavidus pinatubonensis* JMP134, *Paraburkholderia phytofirmans* PsJN and its derivative mutants in indole-3-acetic acid (IAA) or benzoate (Bz).

Strain	IAA	Bz
Paraburkholderia phytofirmans PsJN	**+**	**++**
Cupriavidus pinatubonensis JMP134	**-**	**++**
PsJN::pCR2.1*iacA*	**-**	**++**
PsJN::pCR2.1*iacA iacABIHECDGF-pBS1*	**+**	**++**
PsJN::pCR2.1*iacA iacBIHECDGF-pBS1*	**-**	**++**
PsJN::pCR2.1*iacB*	**-**	**++**
PsJN::pCR2.1*iacB iacABIHECDGF-pBS1*	**+**	**++**
PsJN::pCR2.1*iacB iacAIHECDGF-pBS1*	**-**	**++**
PsJN::pCR2.1*iacC*	**-**	**++**
PsJN::pCR2.1*iacC iacABIHECDGF-pBS1*	**+**	**++**
PsJN::pCR2.1*iacC iacABIHEGF-pBS1*	**-**	**++**
PsJN::pCR2.1*iacD*	**-**	**++**
PsJN::pCR2.1*iacE*	**-**	**++**
PsJN::pCR2.1*iacF*	**-**	**++**
PsJN::pCR2.1*iacG*	**+/-**	**++**
PsJN::pCR2.1*iacH*	**-**	**++**
PsJN::pCR2.1*iacH iacH-pBS1*	**-**	**++**
PsJN::pCR2.1*iacH iacE-pBS1*	**+**	**++**
PsJN::pCR2.1*iacI*	**-**	**++**
PsJN::pCR2.1*iacI iacABIHECDGF-pBS1*	**+**	**++**
PsJN::pCR2.1*iacI iacABHECDGF-pBS1*	**-**	**++**
PsJN::pCR2.1*iacY*	**+**	**++**
PsJN::pCR2.1*iacR1*	**-**	**++**
PsJN::pCR2.1*iacR2*	**-**	**++**
PsJN::pCR2.1*iacS*	**+/-**	**++**
PsJN::pCR2.1*iacT1*	**+**	**++**
PsJN::pCR2.1*catA*	**-***	**++**
PsJN::pCR2.1*iacA2*	**+**	**++**
PsJN::pCR2.1*iacG2*	**+**	**++**
PsJN::pCR2.1*iacT2*	**+**	**++**

++, Growth after 24 h; +, Growth after 48h; +/-, Growth after 60h; -, No growth after 7 days. WT: wild type. IAA 2.5 mM; Bz 2.5 mM. *Growth medium turned brown.

Table S4. ^{1}H and ^{13}C NMR data of compound 1.

Metabolite	Structure	Nucleus	Splitting	Chemical shift (ppm)	Coupling constants (Hz)
2-(3-hydroxy-2-oxoindolin-3-yl)acetic acid		H1	bs	3.42	-
		H2	s	2.91	-
		H3	bs	3.42	-
		H4	d	7.31	*J*=7.3
		H5	t	6.93	*J*=7.5
		H6	t	7.19	*J*=7.6
		H7	d	6.79	*J*=7.7
		H8	s	10.22	-
		C1´	-	178.5	-
		C2´	-	42.2	-
		C3´	-	73.0	-
		C4´	-	131.7	-
		C5´	-	124.3	-
		C6´	-	121.7	-
		C7´	-	129.6	-
		C8´	-	109.9	-
		C9´	-	143.2	-
		C10´	-	170.7	-

Singlet, doublet, triplet and broad signal are abbreviated as s, d, t and bs, respectively. Chemical shift and coupling constants were calculated from representative spectra obtained from compound 1 dissolved in DMSO-*d*6.^{1}H and ^{13}C NMR data were recorded at 400 and 100 MHz, respectively.

Supplemental material references

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