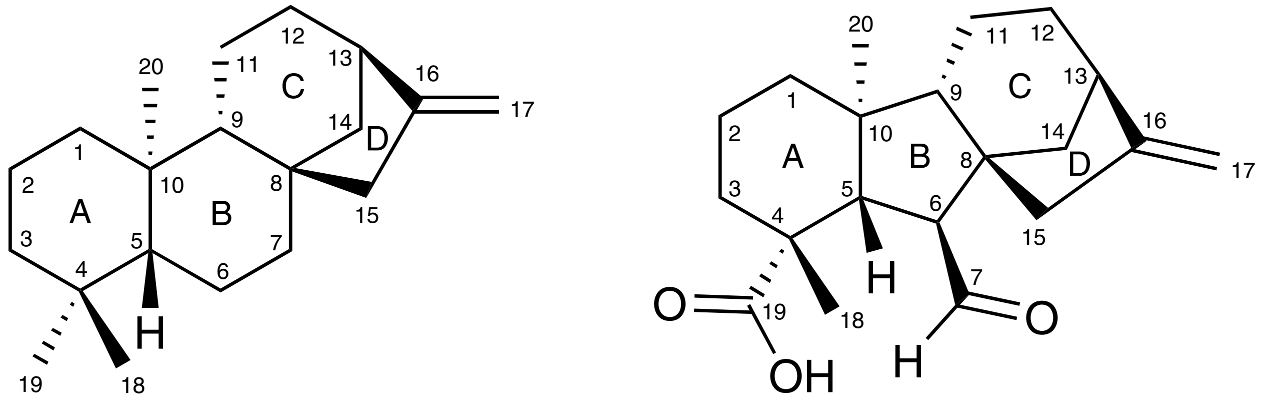
Supplementary Information for:

Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution

Ryan S. Nett[1], Mariana Montanares[2], Ariana Marcassa[4], Xuan Lu[1], Raimund Nagel[1], Trevor C. Charles[4], Peter Hedden[3], Maria Cecilia Rojas[2], Reuben J. Peters[1*]

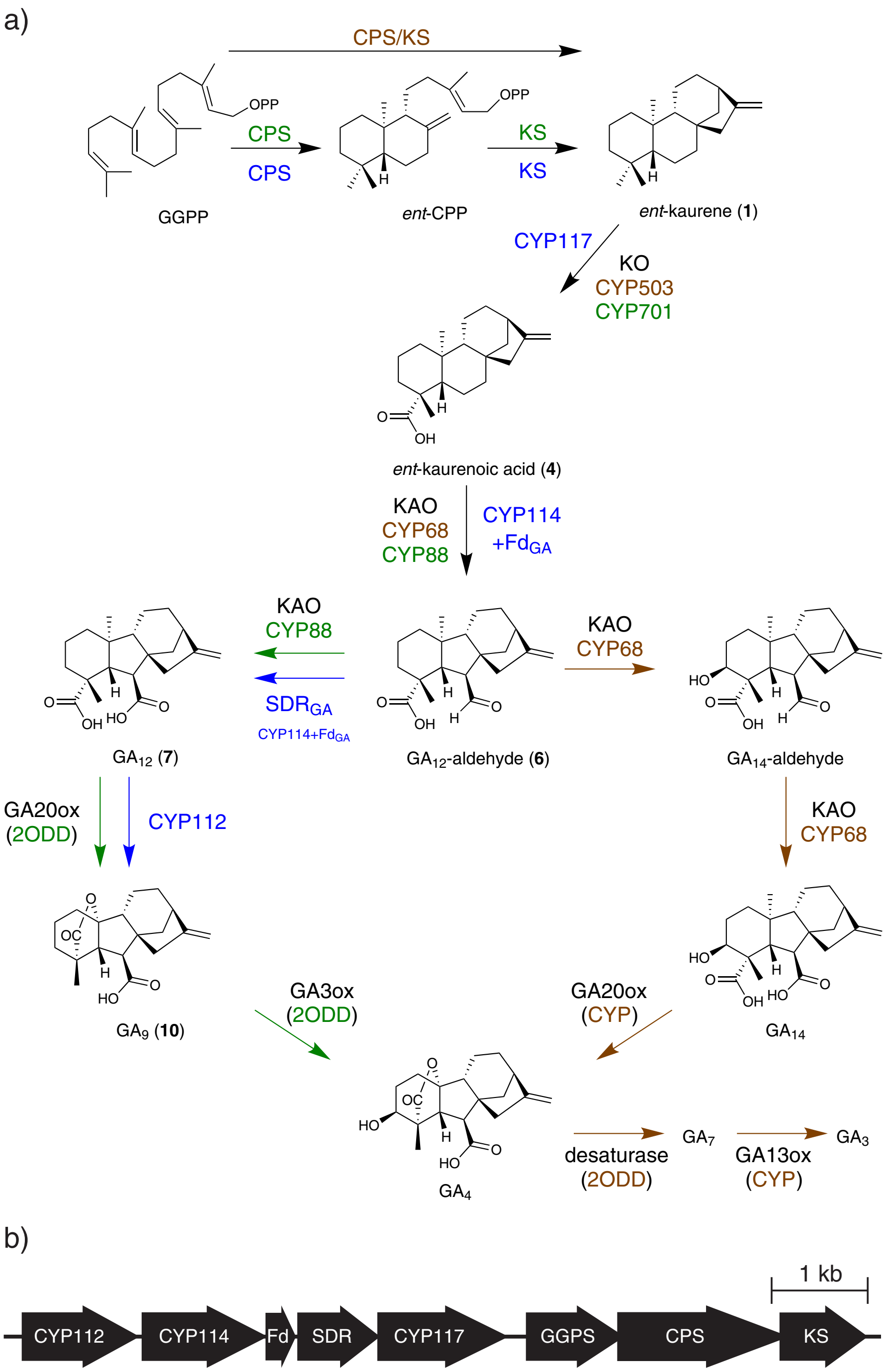
[1]Roy J. Carver Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50011, USA
[2]Laboratorio de Bioorgánica, Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile
[3]Rothamsted Research, Harpenden, Herts AL5 2JQ, United Kingdom
[4]Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada
**Corresponding author. E-mail: rjpeters@iastate.edu*

Supplementary Results – Table of Contents

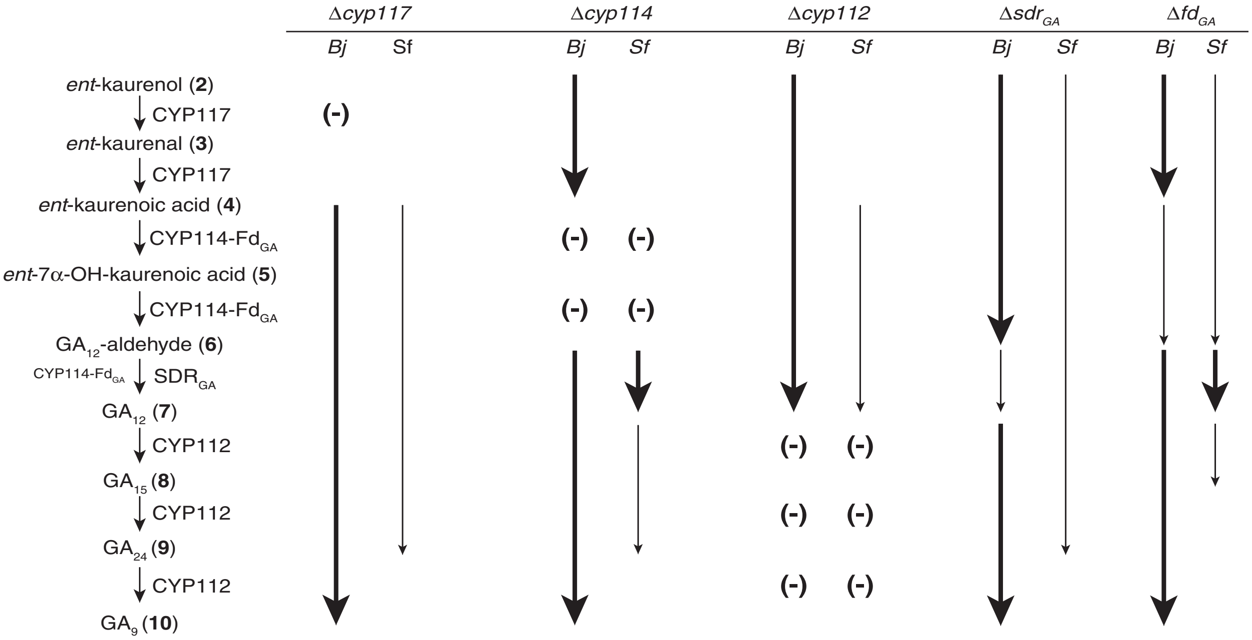


ent-kaurene (**1**)   GA$_{12}$-aldehyde (**6**)

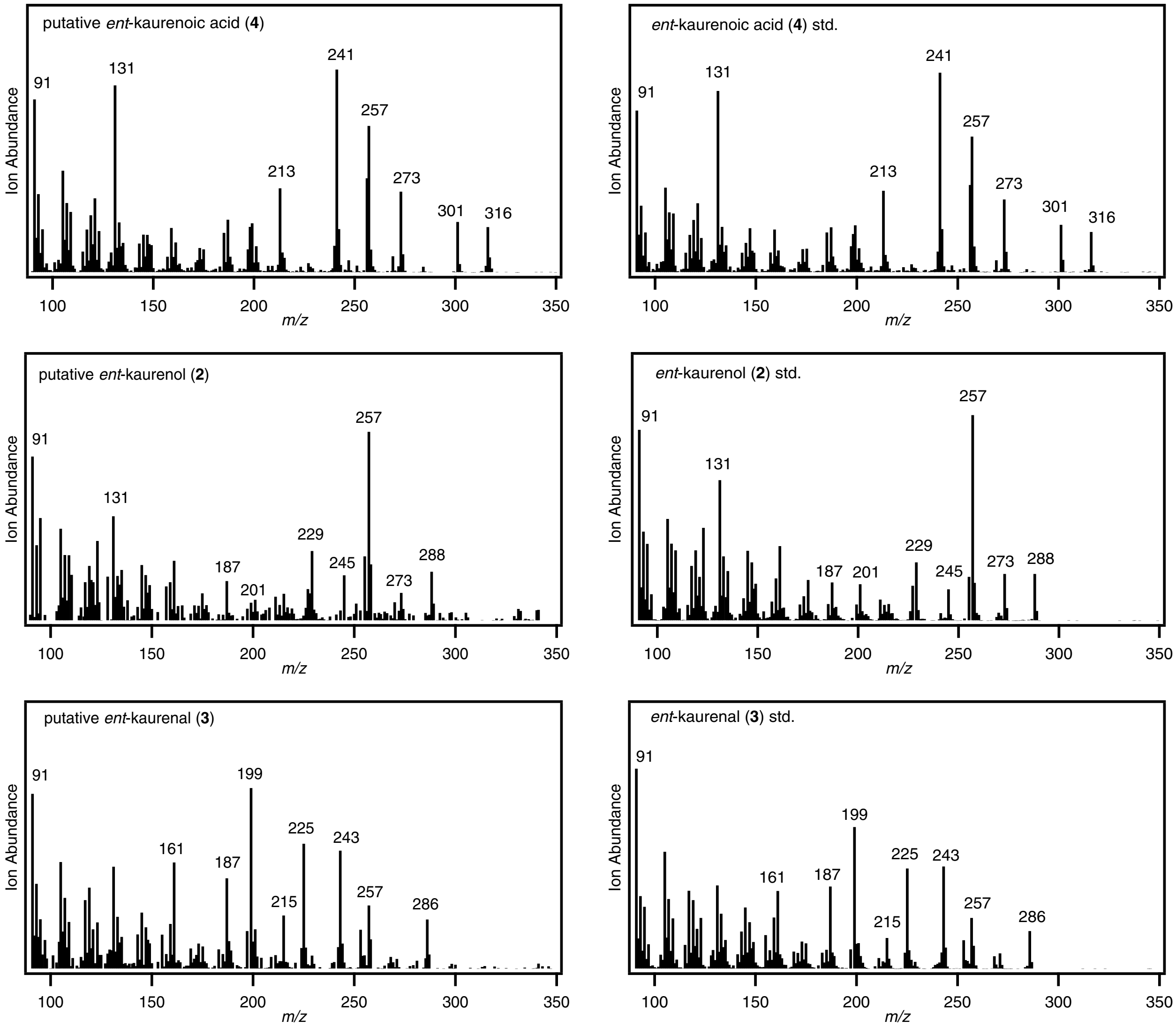
Supplementary Figure 1. Numbering system and ring identification for diterpenoids/gibberellins. Shown is the carbon numbering system for a representative diterpene/diterpenoid, *ent*-kaurene (**1**; *ent*-kaurane backbone, 6-6-6-5), and a representative gibberellin, GA$_{12}$-aldehyde (**6**; *ent*-gibberellane backbone, 6-5-6-5). Also shown is the nomenclature of the characteristic ring systems (A, B, C, and D). Note the changes in **6** that result from B-ring contraction and extrusion of C-7‡. (‡this is the presumed carbon that is extruded, but has not been shown for bacterial GA biosynthesis).



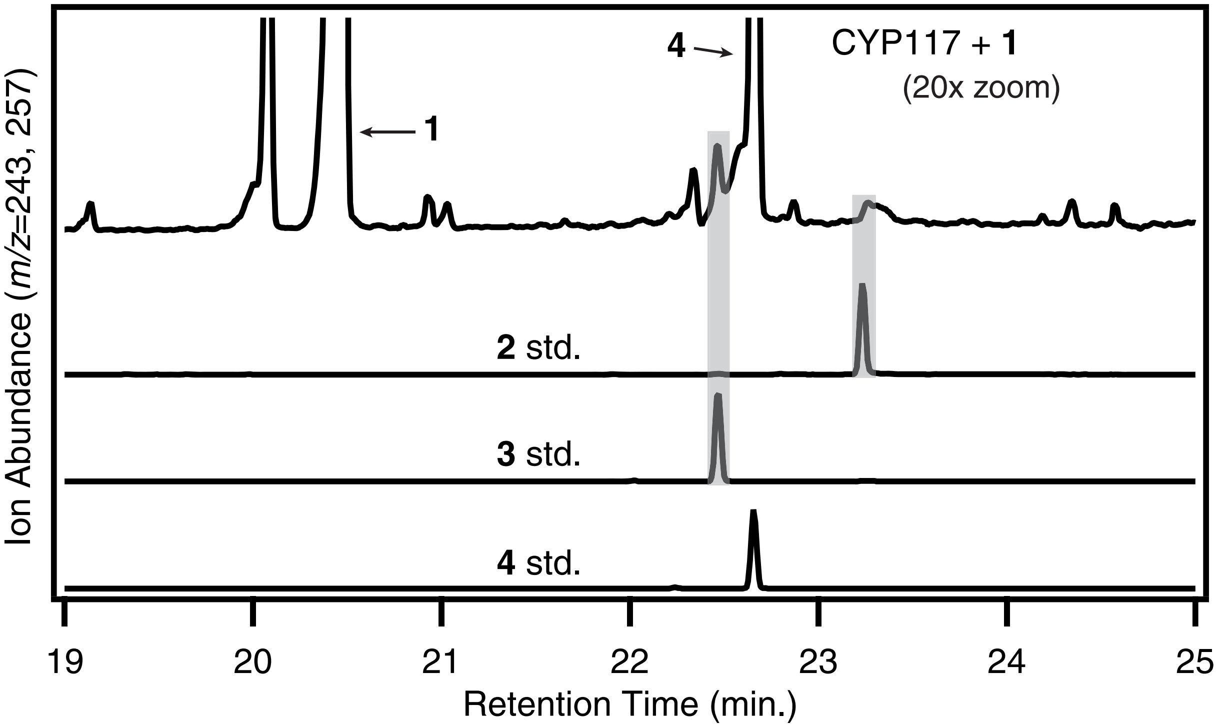
Supplementary Figure 2. GA biosynthesis in plants, fungi, and bacteria. a) Convergent biosynthetic pathways have evolved for GA production in plants (green), fungi (brown), and core-operon containing rhizobia (blue). Shown here are abbreviated pathways, with plants and fungi converging on GA_4. Note that the 13-hydroxylation pathway for plants is not represented here. Also, while GA_3 is the major final product for the fungus *Gibberella fujikuroi*, other fungal species produce different bioactive GAs as their major product. GGPP=(*E,E,E*)-geranylgeranyl diphosphate; GGPS=(*E,E,E*)-geranylgeranyl diphosphate synthase; *ent*-CPP=*ent*-copalyl diphosphate; CPS=*ent*-copalyl diphosphate synthase; KS=*ent*-kaurene synthase; KO=*ent*-kaurene oxidase; KAO=*ent*-kaurenoic acid oxidase; CYP=cytochrome P450 monooxygenase; 2ODD=2-oxoglutarate dependent dioxygenase. b) GA biosynthetic operon found in *B. japonicum and S. fredii*. 1 kb=1 kilobase; Fd=ferredoxin; SDR=short chain dehydrogenase/reductase.



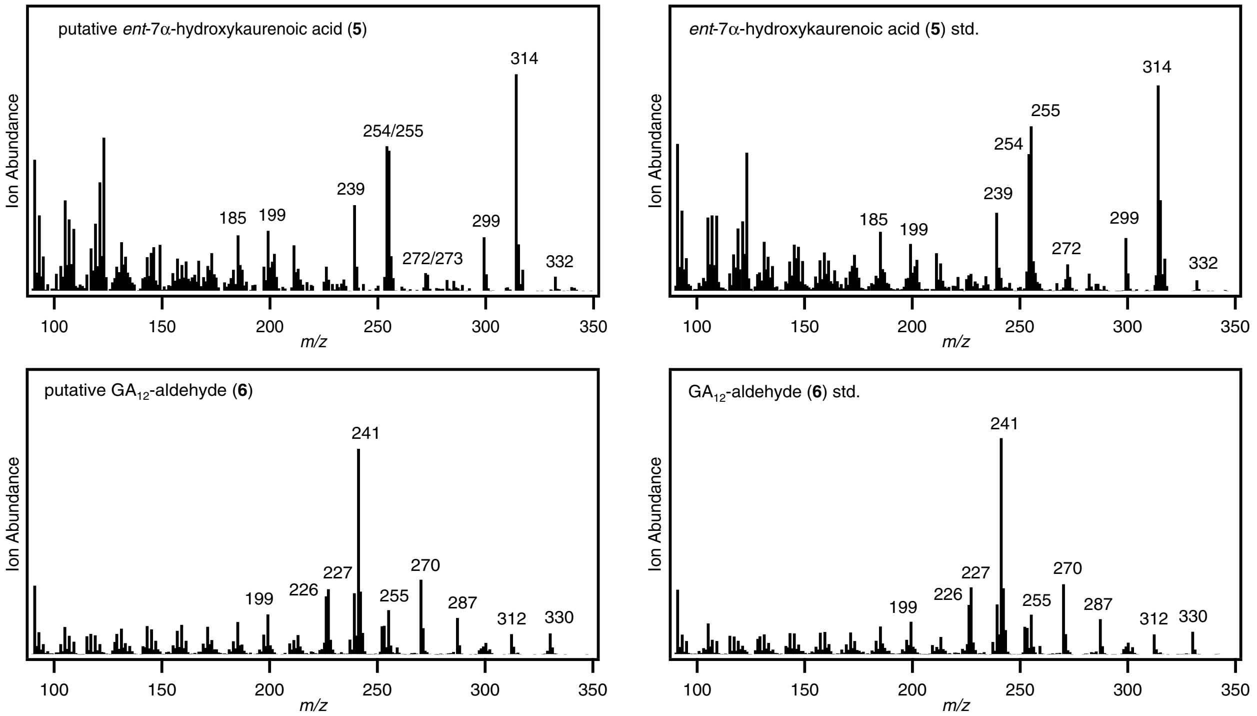
Supplementary Figure 3. Summary of GA biosynthesis reactions detected in *Bradyrhizobium japonicum* and *Sinorhizobium fredii* knockout bacteroid incubations. Arrows indicate observed reactions, with arrow thickness representing relative efficiency of the detected reactions. (-): reaction not detected. Note that overall substrate turnover was higher in *B. japonicum*.



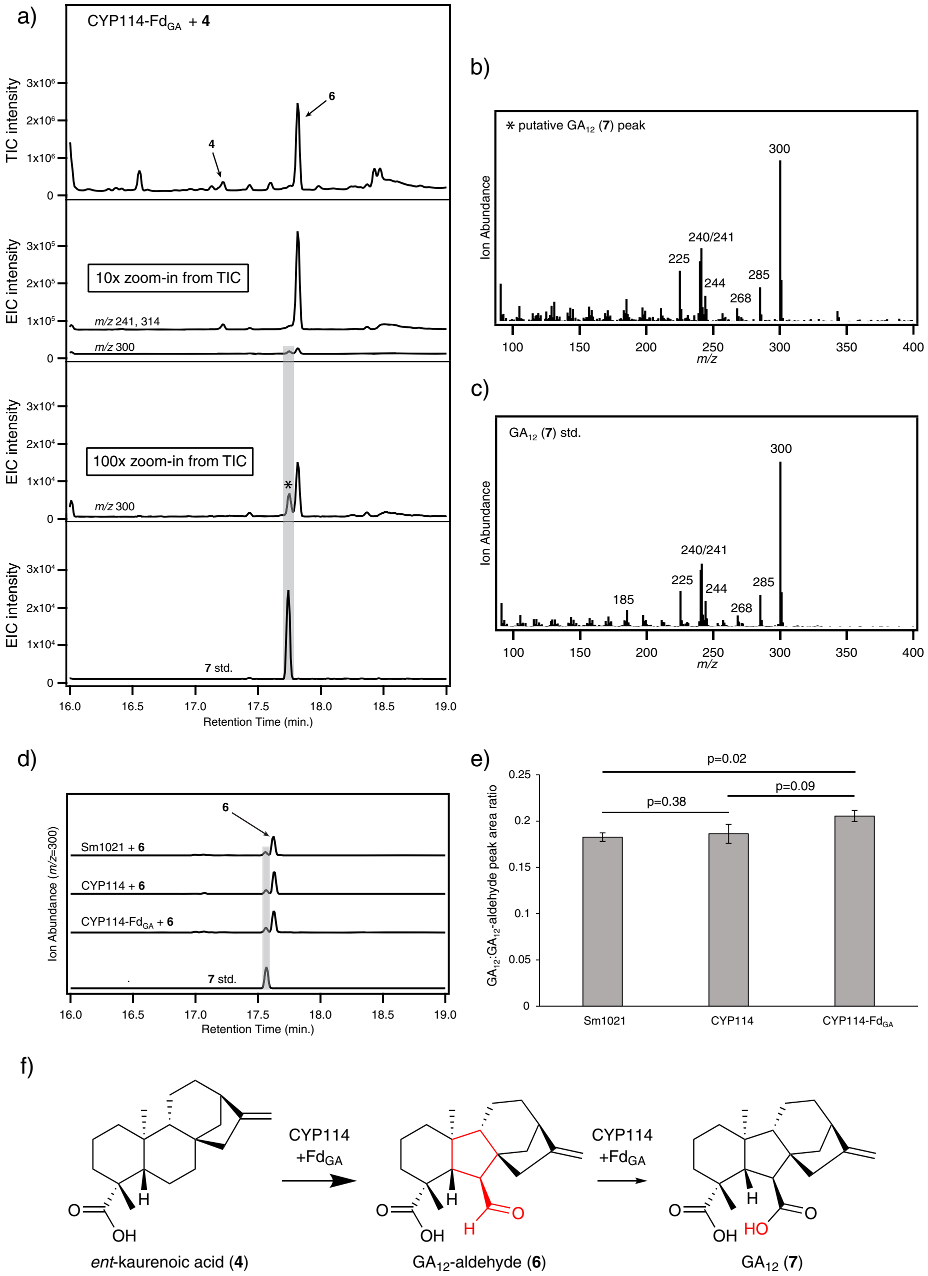
Supplementary Figure 4. Mass spectra of putative *ent*-kaurenol (2), *ent*-kaurenal (3), and *ent*-kaurenoic acid (4) from CYP117 incubations. To confirm production of **2**, **3**, and **4** from incubations of CYP117 with *ent*-kaurene (**1**), **2**, or **3**, the mass spectrum for each compound was compared to that of an authentic standard (std.). These mass spectra correspond to the peaks identified in Figure 3 and Supplementary Figure 5.



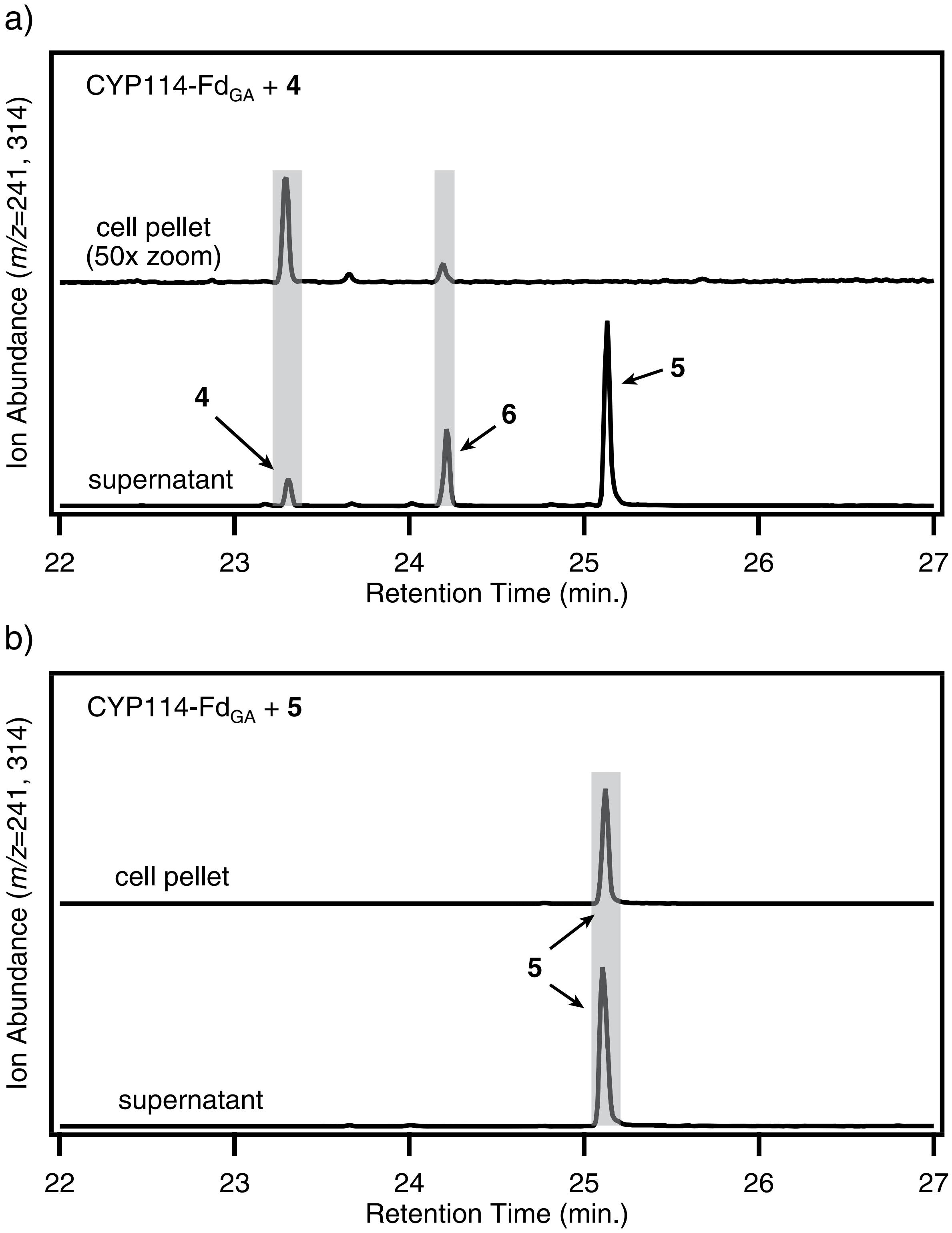
Supplementary Figure 5. Trace amounts of *ent*-kaurenol (2) and *ent*-kaurenal (3) are produced from incubation of *ent*-kaurene (1) with CYP117. In addition to the C-19 acid, *ent*-kaurenoic acid (**4**), the C-19 alcohol (**2**) and aldehyde (**3**) are produced from incubations of **1** with CYP117, as shown here in gas chromatograms with comparison to authentic standards (stds.) of **2**, **3**, and **4**, confirming the sequential oxidation of C-19 catalyzed by CYP117. To obtain sufficient peak resolution for detection of **2** and **3**, the sample shown here was run using a decreased rate of temperature increase, resulting in retention time change from those seen in Figure 3.



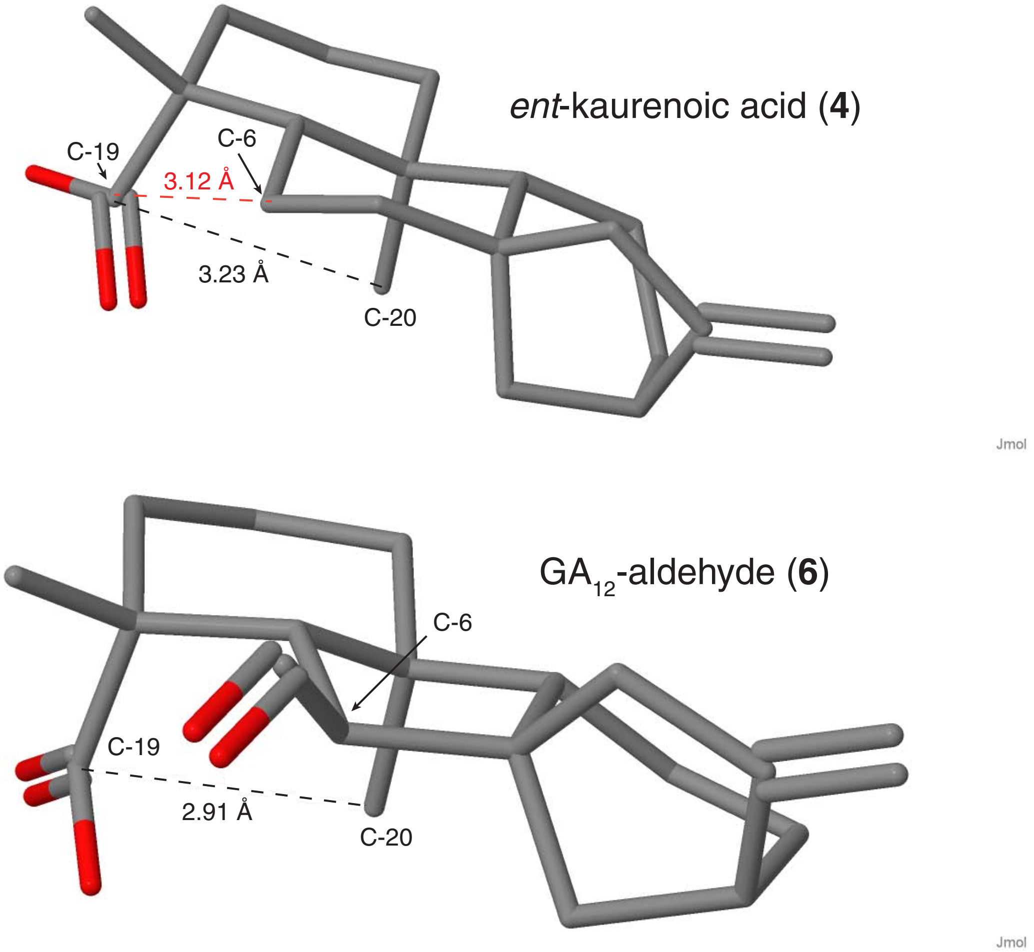
Supplementary Figure 6. Mass spectra of putative *ent*-7α-hydroxykaurenoic acid (5) and GA$_{12}$-aldehyde (6) detected in CYP114 (+/- Fd$_{GA}$) incubations. To confirm production of **5** and **6** from incubations of CYP114 (+/-Fd$_{GA}$) with *ent*-kaurenoic acid (**4**), the mass spectrum for each compound was compared to that of an authentic standard (std.). These mass spectra correspond to the peaks identified in Figure 4.



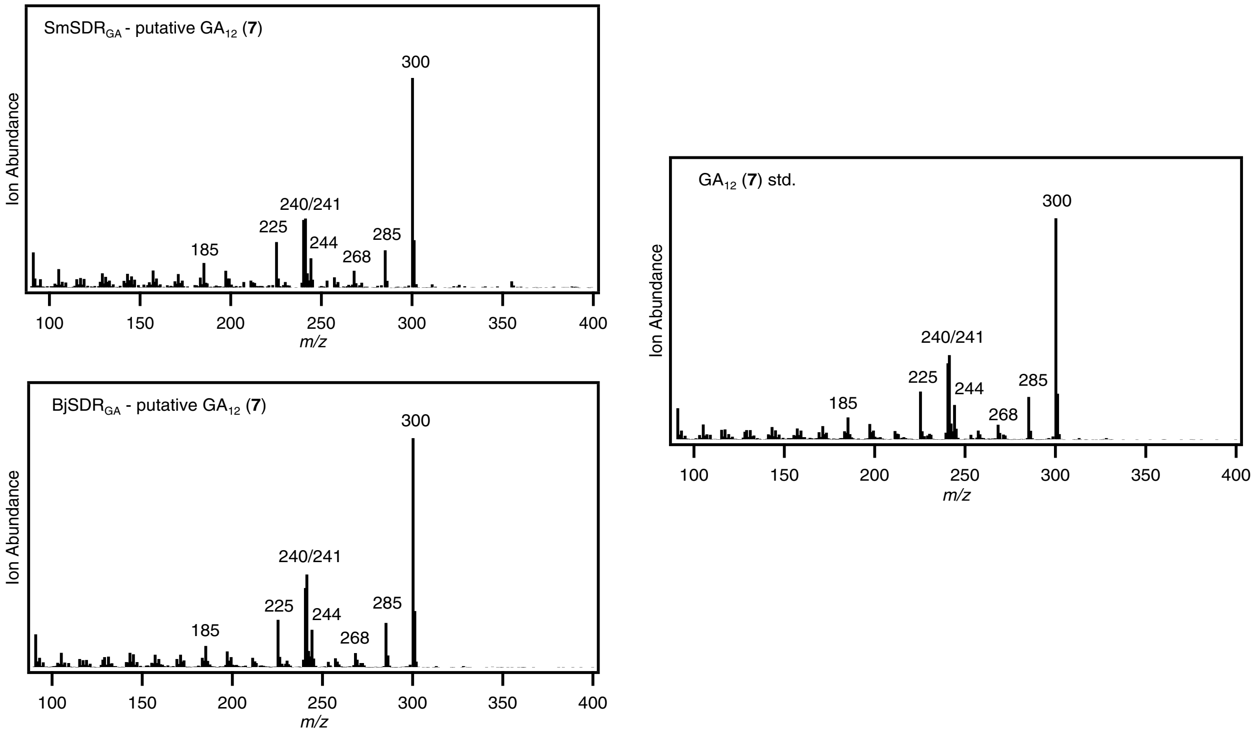
Supplementary Figure 7. CYP114-Fd$_{GA}$ can oxidize GA_{12}-aldehyde (6) to GA_{12} (7) at low efficiency. a) Incubation of *ent*-kaurenoic acid (**4**) in cells expressing CYP114-Fd$_{GA}$ results in trace amounts of a putative GA_{12} (**7**) peak being produced in addition to the much more substantial amounts of GA_{12}-aldehyde (**6**). In particular, **7** is only detectable with compound specific ion extraction (*m/z*=300) and is of low abundance (note the scale changes on the y-axis for each chromatogram; TIC, total ion chromatogram; EIC, extracted ion chromatogram). b) The mass spectrum obtained from the putative **7** peak (*) matches that of c) the mass spectrum from an authentic **7** standard (std.). d) Incubating **6** in cells expressing CYP114-Fd$_{GA}$ results in a small increase in the amount of **7** extracted (**6** substrate contains trace amounts of **7**). e) Quantification of the peak area ratio of **7** to **6** shows that turnover from **6** to **7** is significantly (Student's t-test; $p<0.05$), but only slightly higher in cells expressing CYP114-Fd$_{GA}$; n=3 for each culture, data represent mean values ± standard deviation. f) Thus, while CYP114-Fd$_{GA}$ converts **4** mainly to **6**, **7** is also produced in miniscule amounts. This reduced catalytic efficiency is indicated in this scheme by arrow size.



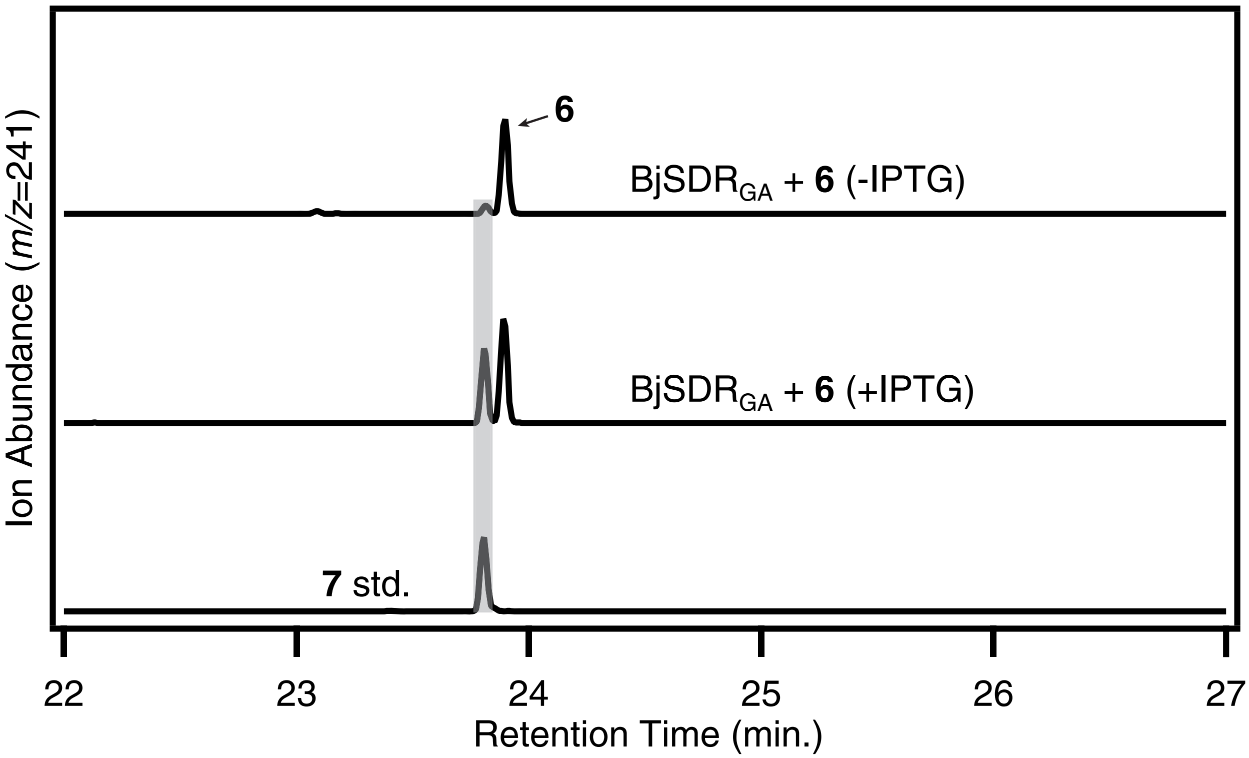
Supplementary Figure 8. *ent*-7α-hydroxykaurenoic acid (5) is sufficiently transported into *S. meliloti* cells heterologously expressing CYP114-Fd$_{GA}$. a) *ent*-kaurenoic acid (**4**), which is converted to *ent*-7α-hydroxykaurenoic acid (**5**) and GA$_{12}$-aldehyde (**6**) by CYP114-Fd$_{GA}$, is found in both the cell pellet and supernatant b) Similarly, **5** is found in both the cell pellet and supernatant, but is unable to be further transformed.



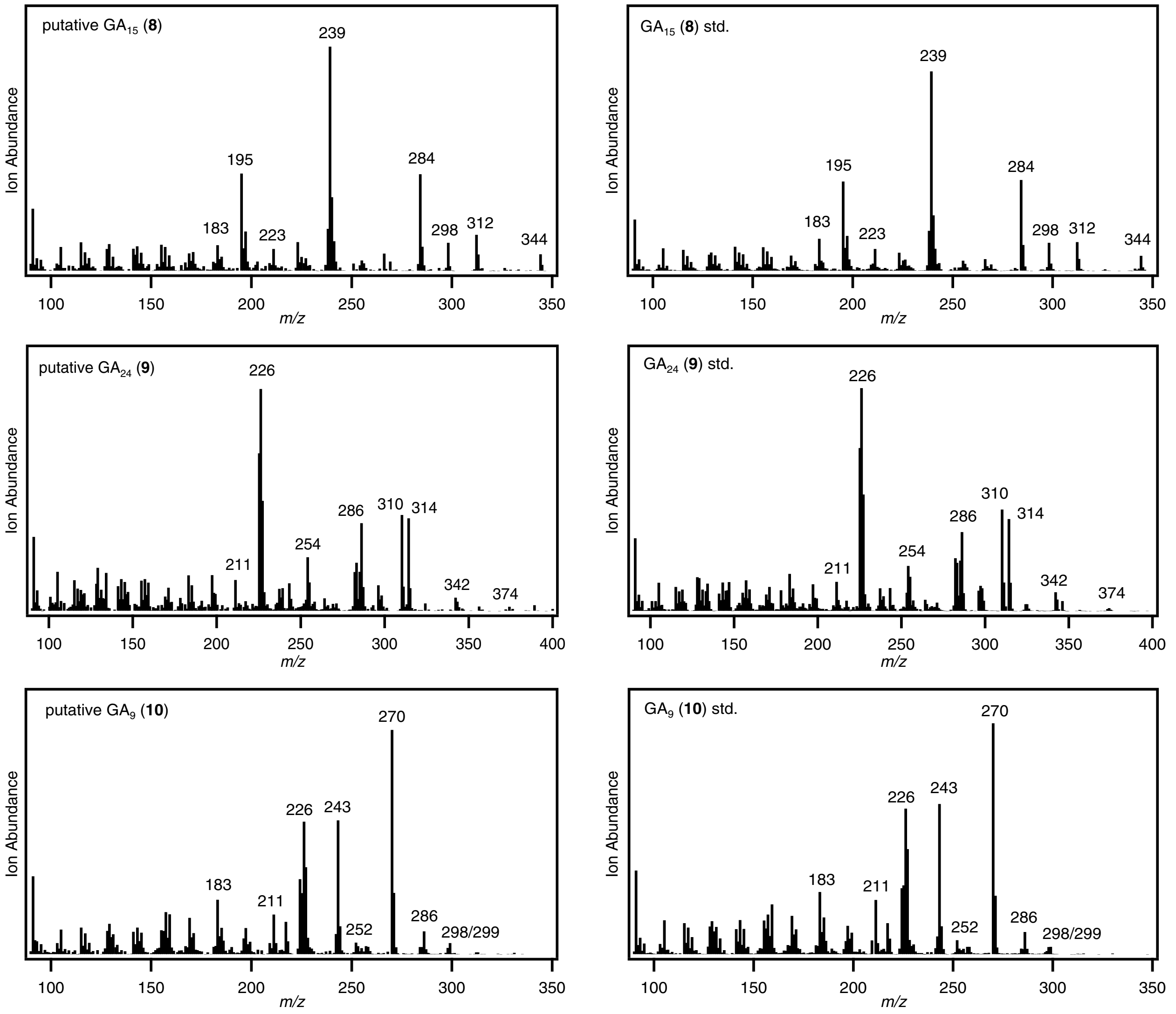
Supplementary Figure 9. Molecular modeling supports the role of a C-19 carboxylate in ring contraction and γ-lactone formation. A three-dimensional rendering of *ent*-kaurenoic acid (**4**) shows that the C-19 is in close proximity (3.12 Å) to C-6, allowing for potential stabilization of a C-6 carbocation by the C-19 carboxylate. Furthermore, B-ring contraction from the conversion of **4** to GA$_{12}$-aldehyde (**6**) brings the C-19 carboxylate 0.32 Å closer to C-20 (from 3.23 Å in **4** to 2.91 Å in **6**) potentially aiding subsequent C-20 loss and lactone formation in the reaction catalyzed by the GA 20-oxidase.



Supplementary Figure 10. Mass spectra of putative GA_{12} (7) from SfSDR$_{GA}$ and BjSDR$_{GA}$ incubations. To confirm production of **7** from incubations of SfSDR$_{GA}$ or BjSDR$_{GA}$ with GA_{12}-aldehyde (**6**), the mass spectrum from each was compared to that of an authentic standard (std.). These mass spectra correspond to the peaks identified in Figure 5 and Supplementary Figure 11.



Supplementary Figure 11. SDR$_{GA}$ from *Bradyrhizobium japonicum* oxidizes GA$_{12}$-aldehyde (6) to GA$_{12}$ (7). When expressed and induced in *E. coli,* BjSDR$_{GA}$ elicits the same function as SDR$_{GA}$ from *S. fredii*, as shown here through gas chromatograms with comparison to an authentic standard (std.) of **7**. IPTG=isopropyl β-D-1-thiogalactopyranoside; used to induce SDR$_{GA}$ expression.



Supplementary Figure 12. Mass spectra of putative GA$_{15}$ (8), GA$_{24}$ (9), and GA$_9$ (10) from CYP112 incubations. To confirm production of **8**, **9**, and **10** from incubations of CYP112 with GA$_{12}$ (**7**), **8**, or **9**, the mass spectrum for each compound was compared to that of an authentic standard (std.). These mass spectra correspond to the peaks identified in Figure 6.

Supplementary Table 1. Products (methyl esters and TMS-ethers) identified by GC-MS from incubations of ^{14}C-labeled and unlabeled GA precursors with bacteroids of *B. japonicum* CYP knock-out mutants.

Mutant	Substrate	Product(s) (% recovered products)	Mass spectrum of Me ester TMSi ether m/z (% relative abundance)
Bj∆cyp117	*ent*-kaurenol	*ent*-kaurenol (100)	M^+ 360(7), 345(8), 270(68), 257(100), 255(27), 241(14), 227(18), 201(17), 187(14), 175(22), 161(24), 147(21), 131(20), 123(68)
Bj∆cyp117	*ent*-$[^{14}C_1]$kaurenoic acid	*ent*-6β,7β-diOH-$[^{14}C_1]$kaurenoic acid (31)	M^+ 594(0.2), 479(85), 404(26), 389(7), 329(7), 269(100), 255(14), 209(36)
		$[^{14}C_1]GA_{15}$ (<1)	M^+ 346(12), 314(10), 286(55), 241(100), 195(28)
		$[^{14}C_1]GA_{12}$ (2)	330(13), 302(100), 300(28), 287(19), 271(13), 243(34), 242(24), 227(26), 187(14)
		GA_{12} norketone (47)	M^+ 362(14), 334(21), 330(100), 302(69), 287(27), 274(21), 271(27), 270(37), 259(48), 255(33), 243(76), 242(39), 227(36), 215(30), 201(36), 199(37), 173(16), 159(24), 145(26)
		GA_{12} norketone standard	M^+ 362(15), 334(18), 330(100), 302(59), 287(24), 274(16), 271(20), 270(27), 259(36), 255(25), 243(62), 242(28), 227(25), 215(21), 201(27), 199(25), 173(10), 159(16), 145(16)
Bj∆cyp117	$[^{14}C_1]GA_{12}$	$[^{14}C_1]GA_{15}$ (8)	M^+ 346(13), 314(21), 286(46), 241(100), 195(21)
		Putative GA_{15} norketone enolTMS (10)	M^+418(100), 403(17), 387(8), 360(21), 359(24), 345(16), 301(11), 270(10), 243(13), 241(17), 237(11), 223(13), 197(10), 157(9), 143(42), 117(34)
		$[^{14}C_1]GA_{24}$ (6)	M^+ 376(3), 344(26), 316(67), 312(28), 288(61), 287(51), 284(36), 256(33), 229(62), 228(100), 227(87)

		Putative GA$_{24}$ norketone enolTMS (21)	M$^+$448(18), 433(5), 419(4), 387(10), 360(10), 359(10), 327(5), 298(11), 271(10), 270(12), 238(23), 223(8), 221(10), 179(7), 168(100), 143(27), 117(10)
		[^{14}C$_1$]GA$_9$ (trace)	M$^+$ 332(4), 300(100), 288(8), 272(93), 243(80), 229(60), 228(53)
		GA$_9$ norketone enolTMS (2)	M$^+$ 404(100), 389(16), 361(24), 360(16), 345(37), 301(27), 287(10), 270(6), 243(9), 211(13), 195(6), 183(9), 159(14), 143(52)
		[^{14}C$_1$]GA$_{12}$ (5)	M$^+$ 362(1), 330(14), 302(100), 287(22), 271(6), 243(31), 242(26), 227(16), 187(11)
		GA$_{12}$ norketone (48)	M$^+$ 362(14), 334(20), 330(100), 302(58), 287(27), 274(20), 271(22), 270(33), 259(43), 255(29), 243(68), 242(33), 227(30), 215(24), 201(31), 199(32), 173(12), 159(20), 145(21)
Bj*Δ*cyp114	*ent*-kaurenol	*ent*-kaurenol (44)	M$^+$ 360(9), 345(8), 270(70), 257(100), 255(24), 241(12), 227(16), 201(14), 187(15), 175(18), 161(20), 147(29), 131(21)
		ent-kaurenal (41)	M$^+$ 286(51), 271(20), 257(46), 253(13), 243(100), 225(22), 215(22), 199(25)
		ent-kaurenoic acid (14)	M$^+$ 316(50), 301(39), 273(70), 257(100), 256(41), 241(70), 201(13), 187(22), 131(37), 121(57)
Bj*Δ*cyp114	*ent*-[^{14}C$_1$]kaurenoic acid	*ent*-7-oxo-[^{14}C$_1$]kaurenoic acid (0.3) (13-OHKA 10%)	M$^+$ 332(55), 300(11), 272(79), 257(33), 175(26), 149(100), 121(31)
Bj*Δ*cyp114	*ent*-7α-OH-[^{14}C$_4$]kaurenoic acid	*ent*-7-oxo-[^{14}C$_4$]kaurenoic acid (66)	M$^+$ 338(51), 336(29), 330(7), 306(7), 278(67), 276(38), 270(9), 263(25), 261(24), 205(10), 181(11), 177(12), 151(100), 149(38), 147(16), 121(27)

BjΔcyp114	[^{14}C$_4$]GA$_{12}$-aldehyde	[^{14}C$_4$]GA$_{12}$ (25)	M$^+$ 368(1), 336(16), 334(13), 328(7), 308(100), 306(69), 300(48), 293(11), 291(16), 285(11), 247(37), 246(37), 241(15), 231(17), 229(17)
		[^{14}C$_4$]GA$_{15}$ (5)	M$^+$ 352(12), 350(8), 344(5), 320(15), 318(11), 306(17), 304(11), 290(44), 288(21), 245(100), 243(39), 239(39), 201(31)199(18)
		[^{14}C$_4$]GA$_{24}$ (35)	M$^+$ 382(3), 380(2), 350(27), 348(15), 322(66), 320(49), 318(45), 314(34), 294(35), 293(47), 292(53), 290(47), 286(30), 262(26), 261(30), 260(26), 233(46), 232(80), 231(100), 229(43), 227(25), 226(28), 225(29)
		[^{14}C$_4$]GA$_9$ (25)	M$^+$ 338(5), 336(4), 306(100), 304(54), 298(33), 294(15), 292(13), 276(90), 274(43), 270(27), 251(47), 249(33), 243(18), 233(60), 232(63), 231(48), 230(40), 227(18), 226(18)
BjΔcyp114	[^{14}C$_1$]GA$_{12}$	[^{14}C$_1$]GA$_{15}$ (13)	M$^+$ 346(16), 314(25), 286(53), 241(100), 195(24)
		[^{14}C$_1$]GA$_{24}$ (56)	M$^+$ 376(5), 344(35), 316(100), 312(42), 288(76), 287(58), 284(41), 256(45), 229(63), 228(91), 227(93)
		[^{14}C$_1$]GA$_9$ (31)	M$^+$ 332(6), 300(100), 288(14), 272(99), 243(50), 229(52), 228(55), 217(28)
BjΔcyp112	*ent*-kaurenol	*ent*-kaurenol (88)	M$^+$ 360(8), 345(8), 270(73), 257(100), 255(25), 241(14), 227(17), 201(15), 187(14), 175(21), 161(23), 147(21), 131(16), 123(65)
		ent-kaurenal (9)	M$^+$ 286(54), 271(17), 257(53), 253(9), 243(100), 225(39), 215(17), 199(33)
		ent-kaurenoic acid (3)	M$^+$ 316(56), 301(38), 273(80), 257(100), 256(34), 241(59), 201(11), 187(21), 131(44), 121(57)

BjΔcyp112	*ent*-$[^{14}C_1]$kaurenoic acid	*ent*-7α-OH$[^{14}C_1]$kaurenoic acid (<1)	M^+ 406(38), 391(12), 316(100), 301(29), 257(57), 256(46), 241(26)
		$[^{14}C_1]GA_{12}$ (37)	M^+ 362((1), 330(20), 302(100), 300(29), 287(18), 271(7), 243(29), 242(24), 227(15), 187(8)
BjΔcyp112	$[^{14}C_1]GA_{12}$	$[^{14}C_1]GA_{12}$ (93)	M^+ 362(1), 330(19), 302(100), 300(14), 287(16), 271(7), 243(27), 242(22), 227(15), 187(8)
		$[^{14}C_1]GA_{12}$ norketone (7)	M^+ 362(21), 334(16), 330(100), 302(72), 287(17), 271(33), 270(22), 259(21), 255(23), 243(44), 242(37), 227(15), 215(37), 201(27), 199(22), 173(17), 159(10), 145(11)
BjΔcyp112	$[^{14}C_1]GA_{15}$	$[^{14}C_1]GA_{15}$ (96)	M^+ 346(14), 314(15), 300(10), 286(43), 241(100), 195(19)
		Putative GA_{15} norketone enolTMS (3)	M^+418(100), 403(19), 387(7), 360(15), 359(23), 345(14), 301(8), 270(10), 243(17), 241(13), 237(9), 223(11), 197(10), 143(42)
		$[^{14}C_1]GA_{24}$ (1)	M^+ 376(6), 344(32), 316(93), 312(29), 288(76), 287(65), 285(33), 284(34), 256(35), 229(64), 228(95), 227(100)
BjΔcyp112	$[^{14}C_1]GA_{24}$	$[^{14}C_1]GA_{24}$ (>99)	M^+ 376(3), 344(31), 316(85), 312(28), 288(76), 287(57), 285(37), 284(33), 256(36), 229(64), 228(92), 227(100)
		Putative GA_{24} norketone (<1)	348(24), 330(17), 320(95), 316(100), 298(27), 288(81), 285(26), 284(27), 273(23), 270(21), 260(27), 256(79), 246(46), 245(40), 229(67), 228(54), 227(64), 213(26), 194(22), 185(51), 168(72)

Supplementary Table 2. Products (methyl esters and TMS-ethers) identified by GC-MS from incubations of ^{14}C-labeled GA precursors with bacteroids of *S. fredii* CYP knock-out mutants.

Mutant	Substrate	Product(s) (% recovered products)	Mass spectrum of Me ester TMSi ether m/z (% relative abundance)
SfΔcyp117	*ent*-[^{14}C$_1$]kaurenoic acid	*ent*-7α-OH[^{14}C$_1$]kaurenoic acid (5)	M^+ 406(44), 391(14), 316(100), 301(28), 257(50), 256(45), 241(41)
		[^{14}C$_1$]GA$_{12}$ (9)	330(15), 302(100), 300(14), 287(18), 271(9), 243(30), 242(18), 227(17), 187(10)
		ent-3-OH[^{14}C$_1$]kaurenoic acid (6)	M^+ 406(5), 391(15), 346(11), 346(11), 316(45), 301(13), 277(89), 257(36), 241(19), 217(8), 189(100)
		ent-15β-OH[^{14}C$_1$]kaurenoic acid (12)	M^+ 406(7), 391(2), 316(11), 301(14), 274(10), 257(26), 241(11), 199(13), 197(11), 158(100)
		ent-[^{14}C$_1$]kaurenoic acid (46)	M^+ 318(55), 303(46), 273(100), 259(93), 258(59), 257(54), 243(83), 213(48), 189(27), 121(97)
		ent-kaurenoic acid norketone (5)	M^+ 318(21), 300(5), 286(19), 259(100), 245(12), 241(12), 218(9), 189(8)
SfΔcyp117	[^{14}C$_1$]GA$_{12}$	[^{14}C$_1$]GA$_{12}$ (65)	M^+ 362(1), 330(17), 302(100), 300(7), 287(17), 271(6), 243(28), 242(22), 227(15), 187(8)
		GA$_{12}$ norketone (6)	M^+ 362(15), 334(18), 330(100), 302(59), 287(24), 274(16), 271(23), 270(35), 259(41), 255(26), 243(59), 242(30), 227(27), 215(23), 201(27), 199(28), 173(11), 159(15), 145(15)
		[^{14}C$_1$]GA$_{15}$ (29)	M^+ 346(15), 314(19), 300(12), 286(47), 241(100), 213(8), 195(21)
		[^{14}C$_1$]GA$_{24}$ (<1)	344(24), 316(81), 312(43), 288(94), 287(61), 285(38), 284(41), 256(54), 229(60), 228(95), 227(100)

SfΔcyp114	*ent*-[$^{14}C_1$]kaurenoic acid	*ent*-[$^{14}C_1$]kaurenoic acid (100)	M$^+$ 318(55), 303(40), 273(100), 259(95), 258(57), 257(47), 243(81), 213(48), 189(24), 121(93)
SfΔcyp114	*ent*-7α-OH-[$^{14}C_4$]kaurenoic acid	*ent*-7α-OH-[$^{14}C_4$]kaurenoic acid (58)	M$^+$ 412(39), 410(47), 408(22), 404(3), 397(14), 395(17), 322(86), 320(100), 318(50), 307(40), 305(44), 263(51), 262(51), 261(70), 260(54), 259(37), 247(38), 245(43), 240(30), 227(29), 225(31)
		7-oxo-[$^{14}C_4$]kaurenoic acid (42)	M$^+$ 338(36), 336(39), 334(20), 330(3), 306(6), 304(7), 278(48), 276(60), 274(31), 263(19), 261(29), 259(17), 205(8), 203(11), 181(14), 179(17), 177(15), 151(100), 149(68), 123(21), 121(32)
SfΔcyp114	[$^{14}C_4$]GA$_{12}$-aldehyde	[$^{14}C_4$]GA$_{12}$ (60)	M$^+$ 368(1), 336(18), 334(10), 328(7), 308(100), 306(61), 300(36), 293(16), 291(14), 285(7), 277(8), 247(32), 246(28), 241(11), 231(16), 229(11)
		[$^{14}C_4$]GA$_{15}$ (38)	M$^+$ 352(15), 350(9), 344(4), 320(18), 318(9), 306(12), 304(8), 290(50), 288(18), 284(15), 245(100), 243(48), 239(31), 201(32), 199(15)
		[$^{14}C_4$]GA$_{24}$ (2)	350(18), 348(17), 322(40), 320(36), 318(47), 314(28), 294(26), 293(48), 292(55), 290(52), 262(39), 261(19), 260(26), 233(60), 232(100), 231(73), 229(36), 226(23)
SfΔcyp114	[$^{14}C_1$]GA$_{12}$	[$^{14}C_1$]GA$_{12}$ (45)	M$^+$ 362(0.6), 330(17), 302(100), 300(8), 287(17), 271(6), 243(27), 242(21), 227(15), 187(8)
		GA$_{12}$ norketone (2)	M$^+$ 362(17), 334(23), 330(100), 302(58), 287(23), 274(12), 271(15), 270(28), 259(36), 255(18), 243(58), 242(28), 227(23), 215(15), 201(22), 199(18), 173(12), 159(19), 148(23)

		$[^{14}C_1]GA_{15}$ (50)	M$^+$ 346(16), 314(18), 300(12), 286(47), 241(100), 213(8), 195(19)
		$[^{14}C_1]GA_{24}$ (3)	344(40), 316(95), 312(44), 288(83), 287(62), 285(29), 284(53), 256(40), 229(49), 228(100), 227(91)
SfΔcyp112	*ent*-$[^{14}C_1]$kaurenoic acid	*ent*-7α-OH-$[^{14}C_1]$kaurenoic acid (10)	M$^+$ 406(46), 391(15), 316(100), 301(32), 257(68), 256(55), 241(32)
		$[^{14}C_1]GA_{12}$ (10)	330(16), 302(100), 287(17), 271(6), 243(27), 242(26), 227(18), 187(10)
		ent-15α-OH$[^{14}C_1]$kaurenoic acid (9)	M$^+$ 406(6), 391(8), 316(8), 301(21), 274(15), 257(37), 241(18), 199(15), 197(15), 158(100)
		7-oxo-$[^{14}C_1]$kaurenoic acid (9)	M$^+$ 332(32), 300(8), 272(66), 257(38), 175(31), 149(100)
		ent-$[^{14}C_1]$kaurenoic acid (35)	M$^+$ 318(56), 303(43), 273(100), 259(92), 258(59), 257(54), 243(87), 213(47), 189(26), 121(99)
SfΔcyp112	$[^{14}C_1]GA_{12}$	$[^{14}C_1]GA_{12}$ (100)	M$^+$ 362(0.5), 330(15), 302(100), 300(7), 287(17), 271(6), 243(28), 242(22), 227(16), 187(8)
SfΔcyp112	$[^{14}C_1]GA_{15}$	$[^{14}C_1]GA_{15}$ (100)	M$^+$ 346(12), 314(15), 300(12), 286(47), 241(100), 213(9), 195(25)
SfΔcyp112	$[^{14}C_1]GA_{24}$	$[^{14}C_1]GA_{24}$ (100)	M$^+$ 376(3), 344(27), 316(76), 312(22), 288(67), 287(51), 285(36), 284(33), 256(35), 229(61), 228(85), 227(100)

Supplementary Table 3. Products (methyl esters and TMS-ethers) identified by GC-MS from incubations of ^{14}C-labeled and unlabeled GA precursors with bacteroids of *B. japonicum* SDR$_{GA}$ or Fd$_{GA}$ knock-out mutants.

Mutant	Substrate	Product(s) (% recovered products)	Mass spectrum of Me ester TMSi ether m/z (% relative abundance)
***Bj*Δ*sdr*$_{GA}$**	*ent*-kaurenol	*ent*-kaurenoic acid (22)	M^+ 316(58), 301(43), 273(75), 257(100), 256(40), 241(73), 201(12), 187(29), 131(46), 121(49)
		ent-7α-OH-kaurenoic acid (6)	M^+ 404(39), 389(20), 314(100), 299(31), 285(23), 255(60), 254(40), 239(27)
		GA$_{12}$-aldehyde (2)	M^+ 330(12), 287(11), 270(48), 253(29), 241(100), 227(8), 199(21)
		GA$_{12}$ (18)	M^+ 360(0.6), 328(21), 300(100), 285(20), 269(7), 241(27), 240(22), 225(16), 185(10)
		GA$_{15}$ (3)	M^+ 344(12), 312(19), 298(13), 284(49), 239(100), 211(8), 195(22)
***Bj*Δ*sdr*$_{GA}$**	*ent*-[$^{14}C_1$]kaurenoic acid (KA)	*ent*-7α-OH[$^{14}C_1$]kaurenoic acid (trace)	M^+ 406(37), 391(34), 316(100), 257(73), 256(45), 241(35)
		ent-6α,7α-diOH-[$^{14}C_1$]kaurenoic acid (5)	479(91), 404(26), 389(7), 329(8), 301(5), 269(100), 255(10), 209(41)
		putative *ent*-6α,7α-di-OH-kaurenoic acid norketone (39)	479(100), 419((5), 404(19), 389(5), 361(10), 329(27), 269(85), 255(14), 209(45)
		Unknown $^{14}C_1$ product 1 (11)	318(52), 314(100), 312(27), 299(17), 290(17), 286(43), 269(24), 268(25), 258(50), 257(89), 241(41), 239(56), 229(75), 227(52), 231(30), 197(24), 195(25), 191(28), 187(31), 185(31), 172(40), 150(61), 138(70)

		Unknown $^{14}C_1$ product 2 (31)	316(20), 287(28), 285(13), 270(28), 253(13), 241(100), 239(31), 227(14), 213(17), 207(15), 197(19), 185(22), 159(24), 157(24)
$Bj\Delta sdr_{GA}$	*ent*-7α-OH [$^{14}C_4$]kaurenoic acid	*ent*-6α,7α-diOH-[$^{14}C_4$]kaurenoic acid (34)	485(79), 483(70), 481(34), 477(17), 410(20), 408(25), 395(17), 393(9), 273(100), 271(59), 269(22), 257(13), 213(41), 207(21)
		[$^{14}C_4$]GA$_{12}$-aldehyde (10)	M$^+$ 338(11), 336(13), 330(5), 293(18), 291(15), 287(9), 278(38), 276(38), 274(22), 270(10), 261(24), 259(11), 247(69), 245(100), 243(45), 241(44), 231(14), 203(12), 199(23)
		[$^{14}C_4$]GA$_{12}$ (3)	336(20), 334(29), 308(100), 306(93), 304(81), 302(40), 300(33), 291(20), 289(22), 287(20), 248(30), 247(14), 246(37), 245(26), 244(27), 243(20), 231(33), 227(25)
		[$^{14}C_4$]GA$_{15}$ (13)	M$^+$ 352(16), 350(12), 344(3), 320(13), 318(19), 306(14), 304(11), 290(47), 288(43), 245(100), 243(97), 239(21), 201(36), 199(27)
		[$^{14}C_4$]GA$_{24}$ (3)	350(23), 348(20), 322(63), 320(55), 318(56), 314(47), 294(26), 293(49), 292(69), 290(74), 288(51), 285(33), 262(30), 233(88), 232(60), 231(100), 229(60), 228(58), 227(51), 226(21), 225(19)
		Unknown [$^{14}C_4$] product 1(15)	324(33), 322(44), 320(100), 318(72), 316(50), 314(15), 312(19), 303(17), 301(14), 292(26), 290(40), 274(18), 272(17), 271(25), 263(53), 261(81), 259(39), 245(39), 243(49), 233(64), 231(85), 229(55), 201(42), 187(44)

		Unknown $[^{14}C_4]$ product 2(15)	322(23), 320(32), 314(8), 291(26), 289(27), 276(16), 274(19), 259(14), 257(18), 245(100), 243(87), 241(33), 239(24), 231(20), 229(18), 203(20), 201(28), 189(22), 187(24)
BjΔsdr$_{GA}$	$[^{14}C_4]$GA$_{12}$-aldehyde	$[^{14}C_4]$GA$_{12}$ (4)	336(24), 334(8), 328(10), 308(100), 306(63), 304(14), 302(37), 300(45), 293(14), 291(17), 247(38), 246(35), 245(21), 244(16), 231(23), 229(15)
		$[^{14}C_4]$GA$_{15}$ (7)	M$^+$ 352(12), 350(7), 344(8), 320(13), 318(7), 306(8), 304(6), 290(37), 288(17), 284(17), 245(100), 243(39), 239(30), 201(25), 199(14)
		$[^{14}C_4]$GA$_{24}$ (15)	350(25), 348(15), 342(9), 322(61), 320(39), 318(27), 316(15), 314(29), 294(30), 293(41), 292(43), 291(51), 290(40), 286(27), 262(22), 261(19), 260(24), 233(50), 232(82), 231(100), 230(38), 229(35), 227(29), 226(22), 225(28)
		$[^{14}C_4]$GA$_9$ (15)	M$^+$ 338(9), 336(4), 330(2), 306(100), 304(60), 298(40), 294(23), 292(18), 276(86), 274(48), 270(40), 251(54), 249(30), 243(32), 233(86), 232(68), 231(63), 230(43), 227(50), 226(46)
		Unknown $[^{14}C_4]$ product 1(29)	M$^+$? 352(6), 348(2), 344(2), 324(45), 322(31), 320(100), 318(57), 316(28), 312(35), 303(20), 301(10), 292(45), 290(28), 274(22), 263(72), 261(53), 259(22), 255(34), 245(41), 243(35), 233(69), 231(57), 229(24), 201(27), 187(30), 152(79), 140(79), 123(81)

		Unknown $[^{14}C_4]$ product 2 (11)	322(32), 320(16), 314(13), 291(32), 289(14), 285(16), 276(19), 274(11), 259(13), 245(100), 243(42), 241(17), 239(39), 233(14), 231(13), 229(11), 203(20), 201(17), 199(11), 189(16), 187(18)
BjΔ***sdr***$_{GA}$	$[^{14}C_1]GA_{12}$	$[^{14}C_1]GA_{24}$ (6)	344(20), 316(81), 312(28), 288(78), 287(50), 285(36), 284(36), 256(36), 229(68), 228(77), 227(100)
		putative GA_{24} norketone (enolTMS)(29)	M^+ 448(10), 348(19), 330(19), 320(85), 316(100), 305(10), 298(19), 288(70), 285(27), 284(28), 273(22), 271(19), 270(20), 260(27), 257(33), 256(76), 246(47), 245(40), 229(84), 228(63), 227(49), 213(22), 201(24), 187(41), 185(48), 168(54)
		GA_9 norketone (44)	304(100), 301(14), 286(9), 272(32), 260(47), 258(21), 245(42), 244(34), 229(74), 228(98), 218(12), 217(14), 213(15), 201(44), 200(25), 199(27), 187(19), 185(86), 183(20), 159(33), 145(41)
		GA_{12} norketone (21)	M^+ 362(16), 334(20), 330(100), 315(8), 302(64), 287(29), 274(20), 271(25), 270(36), 259(46), 255(31), 243(69), 242(36), 227(33), 215(27), 201(35), 199(34), 173(15), 159(23), 145(22)
BjΔ***fd***$_{GA}$	*ent*-kaurenol	*ent*-kaurenoic acid (11)	M^+ 316(47), 301(43), 273(75), 257(100), 256(43), 241(72), 201(13), 187(22), 131(47), 121(85)
		ent-7α-OH-kaurenoic acid (2)	M^+ 404(40), 314(100), 299(31), 255(58), 254(43), 239(20)
		7-oxo-kaurenoic acid (72)	M^+ 330(53), 315(2), 298(6), 270(75), 255(29), 242(9), 227(6), 201(6), 199(6), 187(13), 175(15), 147(100)

		13-OH-kaurenoic acid (7)	M^+ 404(27), 389(5), 345(4), 207(5), 193(100), 180(6), 165(9)
***Bj*Δ*fd*$_{GA}$**	*ent*-$[^{14}C_1]$kaurenoic acid	*ent*-7α-OH-$[^{14}C_1]$kaurenoic acid (10)	M^+ 406(40), 391(12), 316(100), 301(30), 257(59), 256(46), 241(29)
		$[^{14}C_1]$GA$_{15}$ (5)	M^+ 346(13), 314(18), 300(8), 286(39), 241(100), 195(20)
		15-OH-$[^{14}C_1]$-kaurenoic acid (3)	M^+ 406(9), 391(5), 316(5), 301(13), 274(12), 257(23), 241(10), 199(9), 197(10), 158(100)
		7-oxo-$[^{14}C_1]$ kaurenoic acid (60)	M^+ 332(54), 300(6), 272(77), 257(28), 255(14), 244(8), 229(7), 203(6), 201(6), 189(5), 175(21), 149(100)
		Putative 7-oxo-kaurenoic acid norketone (13)	M^+ 332(53), 300(12), 272(67), 257(13), 244(10), 217(9), 209(9), 203(9), 126(34), 121(21), 109(100)
		13-OH-$[^{14}C_1]$- kaurenoic acid (1) *Diluted with endogenous compound*	M^+ 406(30), 404(26), 391(6), 389(4), 348(9), 347(5), 277(2), 195(100), 193(82), 180(2)
***Bj*Δ*fd*$_{GA}$**	*ent*-7α-OH-$[^{14}C_4]$kaurenoic acid	7-oxo-$[^{14}C_4]$ kaurenoic acid (39)	M^+ 338(34), 336(41), 334(21), 330(8), 306(5), 304(6), 278(47), 276(60), 272(16), 270(13), 263(18), 261(29), 259(17), 233(9), 219(9), 205(11), 203(12), 181(13), 180(11), 179(16), 177(18), 175(18), 165(11), 163(14), 151(100), 149(82), 147(34)
		Putative 7-oxo-$[^{14}C_4]$kaurenoic acid norketone (4)	M^+ [340(36), 338(37), 336(23), 332(28)], 280(41), 278(63), 276(25), 272(37), 265(12), 263(13), 261(14), 221(16), 215(10), 213(14), 209(16), 169(31), 165(24), 140(35), 125(38), 121(26), 113(94), 111(90), 109(100)

		Putative dehydro-*ent*-7α-OH-[$^{14}C_4$]kaurenoic acid (20)	M$^+$ 410(79), 408(100), 406(50), 404(25), 402(25), 395(20), 393(26), 391(14), 379(6), 377(5), 367(7), 365(8), 351(17), 349(23), 335(33), 333(43), 331(23), 320(16), 318(14), 307(15), 305(14), 293(24), 291(26), 280(25), 278(54), 277(61), 276(27), 275(46), 265(20), 263(21), 247(27), 235(33), 233(25)
		Plus other unidentified products – possibly norketones of the major products as the enol-TMS derivatives	
BjΔfd$_{GA}$	[$^{14}C_4$]GA$_{12}$-aldehyde	[$^{14}C_4$]GA$_{12}$ (4)	336(15), 334(8), 328(8), 308(100), 306(74), 304(13), 300(45), 293(14), 291(20), 247(29), 246(32), 245(21), 244(20), 231(14), 229(10)
		[$^{14}C_4$]GA$_{15}$ (3)	M$^+$ [352(11), 350(7), 344(2)], 320(16), 318(7), 306(6), 304(10), 290(40), 288(14), 245(100), 243(40), 239(32), 201(22), 199(10)
		[$^{14}C_4$]GA$_{24}$ (8)	350(21), 348(18), 342(8), 322(69), 320(38), 318(27), 316(12), 314(19), 294(32), 293(55), 292(51), 291(37), 290(45), 286(17), 262(15), 261(18), 260(26), 233(58), 232(70), 231(100), 230(39), 229(41), 227(20), 226(23), 225(41)
		[$^{14}C_4$]GA$_9$ (4)	M$^+$ 338(7), 306(83), 304(66), 298(35), 294(14), 292(18), 276(100), 274(44), 270(37), 251(62), 249(27), 243(26), 233(70), 232(79), 231(60), 230(53), 227(27), 226(37)
		[$^{14}C_4$]GA$_9$ norketone (enolTMS) (6)	M$^+$ [412(100), 410(60), 404(31)], 397(13), 381(5), 368(17), 367(26), 365(11), 351(37), 349(14), 345(18), 307(20), 305(14), 293(12), 257(13), 217(12), 215(17), 195(14), 161(12), 159(13), 147(25), 145(68), 143(38)

		Putative $[^{14}C_4]GA_{15}$ norketone (enolTMS) (2)	M$^+$ [426(100), 424(62), 418(27)], 409(12), 403(15), 366(21), 365(32), 351(17), 349(18), 345(14), 305(16), 249(19), 247(14), 145(62), 143(53)
		Putative $[^{14}C_4]GA_{24}$ norketone (enolTMS) (16)	M$^+$ [456(15), 454(9), 448(3)], 441(5), 396(8), 366(9), 356(9), 338(8), 336(10), 328(36), 326(24), 324(42), 322(22), 320(19), 296(25), 294(30), 292(19), 262(27), 239(25), 235(33), 233(36), 225(19), 191(29), 170(100), 168(39), 145(38)
		$[^{14}C_4]GA_{12}$ norketone (29)	M$^+$ 370(19), 368(11), 362(5), 342(22), 340(14), 338(100), 336(57), 330(29), 310(58), 308(39), 302(17), 295(21), 292(21), 279(26), 278(37), 276(24), 267(36), 265(31), 262(34), 261(26), 249(80), 248(43), 247(39), 243(21), 233(32), 221(28), 207(30), 205(46)
Bj∆fd$_{GA}$	$[^{14}C_1]GA_{12}$	$[^{14}C_1]GA_{12}$ (33)	M$^+$ 362(0.5), 330(15), 302(100), 287(17), 271(6), 243(26), 242(20), 227(14), 187(7)
		GA_{12} norketone (23)	M$^+$ 362(14), 334(20) 330(100), 302(58), 287(25), 274(16), 271(23), 270(29), 259(38), 255(27), 243(62), 242(29), 227(25), 215(23), 201(29), 199(26), 173(11), 159(17), 145(17)
		$[^{14}C_1]GA_{15}$ (10)	M$^+$ 346(16), 314(16), 300(12), 286(44), 241(100), 195(20)
		Putative GA_{15} norketone enolTMS (3)	M$^+$418(100), 403(20), 387(6), 360(20), 359(25), 345(17), 301(10), 270(10), 243(19), 241(13), 238(8), 223(13), 197(10), 157(7), 143(44), 117(38)

[^{14}C$_1$]GA$_{24}$ (7)	M$^+$ 376(3), 348(11), 344(34), 316(86), 312(32), 288(81), 287(60), 285(32), 284(38), 256(40), 229(76), 228(98), 227(100)
Putative GA$_{24}$ norketone enolTMS (4)	M$^+$448(15), 433(4), 419(4), 387(7), 360(10), 359(8), 327(4), 298(9), 271(8), 270(7), 238(23), 223(7), 221(10), 179(7), 168(100), 143(27), 117(10)
[^{14}C$_1$]GA$_9$ (3)	M$^+$ 332(6), 300(100), 288(18), 272(95), 244(24), 243(57), 229(64), 228(74), 227(30), 218(22), 217(33), 213(18), 185(21), 183(23), 159(39)
GA$_9$ norketone (enolTMS) (1)	M$^+$ 404(100), 389(15), 373(7), 361(26), 360(14), 345(38), 301(18), 287(5), 270(7), 257(10), 243(10), 211(7), 183(4), 159(5), 143(46)

Supplementary Table 4. Products (methyl esters and TMS-ethers) identified by GC-MS from incubations of ^{14}C-labeled and unlabeled GA precursors with bacteroids of *S. fredii* SDR$_{GA}$ or Fd$_{GA}$ knock-out mutants.

Mutant	Substrate	Product (% recovered products)	Mass spectrum of Me ester TMSi ether m/z (% relative abundance)
SfΔsdr$_{GA}$	*ent*-kaurenol	*ent*-kaurenol (95)	M^+ 360(9), 345(9), 270(88), 257(100), 255(30), 241(16), 227(19), 201(17), 187(15), 175(24), 161(27), 147(27), 131(16), 123(77)
		ent-kaurenal (3)	M^+ 286(62), 271(19), 257(57), 252(17), 243(100), 225(36), 215(23), 199(38), 161(43)
		ent-kaurenoic acid (2)	M^+ 316(50), 301(38), 273(67), 257(100), 256(45), 241(74), 201(15), 187(24), 131(41), 121(64)
		ent-7α-OH-kaurenoic acid (<1)	M^+ 404(40), 314(100), 299(21), 255(64), 254(33), 239(25)
SfΔsdr$_{GA}$	*ent*-[$^{14}C_1$]kaurenoic acid (KA)	*ent*-7α-OH-[$^{14}C_1$]kaurenoic acid (18)	M^+ 406(39), 391(14), 316(100), 301(32), 257(56), 256(44), 241(29)
		[$^{14}C_1$]GA$_{12}$ (<1)	330(28), 302(100), 287(49), 271(25), 243(65), 242(37), 227(4), 187(21)
		15-OH-[$^{14}C_1$]KA (7)	M^+ 406(8), 391(7), 316(7), 301(21), 274(11), 257(36), 241(18), 199(7), 197(7), 158(100)
		ent-[$^{14}C_1$]kaurenoic acid (56)	M^+ 318(60), 303(45), 273(100), 259(99), 258(56), 257(52), 243(83), 213(39), 189(26), 121(93)
SfΔsdr$_{GA}$	[$^{14}C_4$]GA$_{12}$-aldehyde	[$^{14}C_4$]GA$_{12}$ (17)	336(16), 334(6), 328(11), 308(100), 306(62), 304(11), 302(9), 300(41), 293(5), 291(8), 246(26), 231(10), 229(15)

		[^{14}C$_4$]GA$_{12}$-aldehyde (60)	M$^+$ 338(12), 336(6), 293(15), 291(7), 287(6), 278(47), 276(28), 270(14), 261(15), 259(6), 247(100), 245(65), 243(20), 241(31), 233(12), 231(9), 203(11)
SfΔsdr$_{GA}$	[^{14}C$_1$]GA$_{12}$	[^{14}C$_1$]GA$_{15}$ (33)	M$^+$ 346(17), 314(16), 300(13), 286(48), 241(100), 195(22)
		[^{14}C$_1$]GA$_{24}$ (4)	344(33), 316(82), 312(31), 288(83), 287(60), 285(51), 284(35), 256(39), 229(68), 228(84), 227(100)
		[^{14}C$_1$]GA$_{12}$ (60)	M$^+$ 362(0.6), 330(17), 302(100), 287(18), 271(7), 243(29), 242(22), 227(16), 187(8)
		GA$_{12}$ norketone (2)	M$^+$ 362(22), 334(22), 330(100), 302(70), 287(30), 274(24), 271(20), 270(45), 259(45), 255(22), 243(76), 242(37), 227(28), 215(24), 201(32), 199(32), 173(11), 159(20), 145(22)
SfΔfd$_{GA}$	*ent*-kaurenol	*ent*-kaurenol (98)	M$^+$ 360(10), 345(9), 270(100), 257(83), 255(31), 241(17), 227(20), 201(18), 187(16), 175(26), 161(27), 147(27), 131(18), 123(81)
		ent-kaurenal (<1)	M$^+$ 286(59), 271(46), 257(45), 243(100), 225(38), 199(9), 161(21)
		ent-kaurenoic acid (1)	M$^+$ 316(48), 301(38), 273(63), 257(100), 256(38), 241(68), 213(27), 201(12), 187(21), 131(31), 121(55)
SfΔfd$_{GA}$	*ent*-[^{14}C$_1$]kaurenoic acid	*ent*-7α-OH-[^{14}C$_1$]kaurenoic acid (2)	M$^+$ 406(68), 316(100), 301(49), 257(29), 256(31), 241(36)
		15-OH-[^{14}C$_1$]-KA (13)	M$^+$ 406(7), 391(5), 316(5), 301(17), 274(12), 257(34), 241(16), 199(10), 197(11), 158(100)
		ent-[^{14}C$_1$]kaurenoic acid (60)	M$^+$ 318(65), 303(44), 273(99), 259(100), 258(60), 257(48), 243(79), 213(40), 189(25), 121(83)

SfΔfd$_{GA}$	*ent*-7α-OH-$[^{14}C_4]$kaurenoic acid	*ent*-7α-OH $[^{14}C_4]$kaurenoic acid (81)	M$^+$ 412(43), 410(46), 408(20), 397(15), 395(16), 322(87), 320(100), 318(45), 307(35), 305(37), 303(18), 278(10), 26350), 262(48), 261(60), 260(52), 259(33), 258(21), 247(38), 245(38), 239(25), 227(30), 189(34)
		$[^{14}C_4]$GA$_{12}$ (8)	336(11), 334(7), 328(11), 308(100), 306(78), 304(7), 300(52), 293(6), 291(8), 247(45), 246(42), 231(35)
SfΔfd$_{GA}$	$[^{14}C_4]$GA$_{12}$-aldehyde	$[^{14}C_4]$GA$_{12}$ (63)	336(26), 334(20), 308(96), 306(100), 304(29), 300(90), 293(23), 291(23), 247(88), 246(67), 244(48), 231(78), 229(15)
		$[^{14}C_4]$GA$_{15}$ (37)	M$^+$ 352(13), 350(8), 320(24), 312(15), 306(15), 290(73), 288(27), 245(100), 243(93), 239(83), 201(72), 199(44)
SfΔfd$_{GA}$	$[^{14}C_1]$GA$_{12}$	$[^{14}C_1]$GA$_{12}$ (78)	330(19), 302(100), 300(24), 287(46), 271(27), 243(57), 242(56), 227(37), 187(31)
		$[^{14}C_1]$GA$_{15}$ (22)	M$^+$ 346(6), 314(20), 300(14), 286(44), 241(100), 195(31)

Supplementary Table 5. Comprehensive examination of enzyme functionality with GA pathway intermediates. **+**, viable substrate; **-**, non-viable substrate; **P**, product formed; n.t., not tested. Note that some intermediates are both products and viable substrates.

	CYP117	CYP114	CYP114-Fd$_{GA}$	SDR$_{GA}$	CYP112
ent-kaurene (**1**)	**+**	**-**	**-**	**-**	**-**
ent-kaurenol (**2**)	**+/P**	**-**	**-**	**-**	**-**
ent-kaurenal (**3**)	**+/P**	**-**	**-**	**-**	**-**
ent-kaurenoic acid (**4**)‡	**-/P**	**+**	**+**	**-**	**-**
ent-7α-OH-kaurenoic acid (**5**)	**-**	**-/P**	**-/P**	**-**	**-**
GA$_{12}$-aldehyde (**6**)	**-**	**-**	**+/P**	**+**	**-**
GA$_{12}$ (**7**)	**-**	**-**	**-/P**	**-/P**	**+**
GA$_{15}$ (**8**)	**-**	**-**	**-**	**-**	**+/P**
GA$_{24}$ (**9**)	**-**	**-**	**-**	**-**	**+/P**
GA$_{9}$ (**10**)	**-**	**-**	**-**	**-**	**-/P**

‡methyl ester derivative was also tested with CYP114 +/-Fd$_{GA}$ with no turnover detected.

Supplementary Table 6. Identity between plant, fungal, and bacterial cytochromes P450 involved in GA biosynthesis. Representative protein sequences from *B. japonicum* USDA110, *Arabidopsis thaliana*, and *Gibberella fujikuroi* were compared using pairwise alignments.

	BjCYP117	**BjCYP114**	**BjCYP112**	**AtKO (CYP701)**	**AtKAO1 (CYP88)**	**AtKAO2 (CYP88)**	**GfKO (CYP503)**	**GfKAO (CYP68A)**	**GfGA20ox (CYP68B)**
BjCYP117	-								
BjCYP114	22.9	-							
BjCYP112	19.8	27	-						
AtKO (CYP701)	18.2	16.4	16.6	-					
AtKAO1 (CYP88)	19	21.8	19.5	19.7	-				
AtKAO2 (CYP88)	16.2	17.5	18.6	19.7	76.2	-			
GfKO (CYP503)	16.4	19.5	17.2	18.2	17.4	16.8	-		
GfKAO (CYP68A)	15.3	13.1	17.6	17.3	20.7	20.7	28	-	
GfGA20ox (CYP68B)	14.3	13.6	17.2	21.4	20.9	19.2	27.7	37.4	-

Supplementary Table 7. List of prominent strains, plasmids, and constructs used in experiments.

Strain, plasmid, construct	Description	Reference or Source
Bacterial Strains		
One Shot® TOP10 Chemically Competent *E. coli*	F- *mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 Δ lacX74 recA1 araD139 Δ(araleu)7697 galU galK rpsL (StrR) endA1 nupG*; for plasmid propagation and cloning	Thermo Fisher Scientific
OverExpress^{TM} C41(DE3) Chemically Competent *E. coli*	F- *ompT hsdSB (rB- mB-) gal dcm* (DE3); for pCDF-BjSDR/Fd expression	Lucigen
One Shot® BL21 Star^{TM} (DE3) Chemically Competent *E. coli*	F- *ompT hsdSB (rB-, mB-) galdcmrne131* (DE3); for pET101-BjCYP114 and pCDF-FdR$_x$/Fd coespression	Thermo Fisher Scientific
C41 pCDF-BjSDR/Fd	*E. coli* C41 cells transformed for expression of *B. japonicum* USDA110 SDR$_{GA}$ expression; Sp^R	This study
BL21 pET101-BjCYP114	*E. coli* BL21 cells transformed for expression of *B. japonicum* USDA110 CYP114	This study
BL21 pET101-BjCYP114+pCDF-FdR$_x$/Fd	*E. coli* BL21 cells transformed for coexpression of *B. japonicum* USDA110 CYP114, Fd$_{GA}$, and FdR1-5	This study
E. coli MM294A	*pro-82 thi-1 endA hsdR17 supE44;* donor strains for triparental mating	51
E. coli MT616	MM294A *recA56* carrying plasmid pRK600, Cm^R; helper strains for triparental mating	52
Sinorhizobium meliloti 1021 (a.k.a. *Ensifer* meliloti 1021, *S. meliloti* Rm1021)	Rhizobial symbiont of *Medicago spp.;* Sm^R; heterologous host for expression of *S. fredii* GA operon genes	46
*Sm*1021 pstb-LAFR5-SfCYP117	*S. meliloti* 1021 transformed for expression of *S. fredii* NGR234 CYP117; Sm^R, Tc^R	This study
*Sm*1021 pstb-LAFR5-SfCYP117-Fd	*S. meliloti* 1021 transformed for coexpression of *S. fredii* NGR234 CYP117 and Fd$_{GA}$; Sm^R, Tc^R	This study
*Sm*1021 pstb-LAFR5-SfCYP114	*S. meliloti* 1021 transformed for expression of *S. fredii* NGR234 CYP114; Sm^R, Tc^R	This study
*Sm*1021 pstb-LAFR5-SfCYP114-Fd	*S. meliloti* 1021 transformed for coexpression of *S. fredii* NGR234 CYP114 and Fd$_{GA}$; Sm^R, Tc^R	This study

*Sm*1021 pstb-LAFR5-SfSDR	*S. meliloti* 1021 transformed for expression of *S. fredii* NGR234 $\mathrm{SDR_{GA}}$; $\mathrm{Sm^R}$, $\mathrm{Tc^R}$	This study
*Sm*1021 pstb-LAFR5-SfCYP112	*S. meliloti* 1021 transformed for expression of *S. fredii* NGR234 CYP112; $\mathrm{Sm^R}$; $\mathrm{Tc^R}$	This study
*Sm*1021 pstb-LAFR5-SfCYP112-Fd	*S. meliloti* 1021 transformed for coexpression of *S. fredii* NGR234 CYP112 and $\mathrm{Fd_{GA}}$; $\mathrm{Sm^R}$, $\mathrm{Tc^R}$	This study
Bradyrhizobium japonicum USDA110 (a.k.a. *B. diazoefficiens* USDA110)	wild-type rhizobial symbiont of *Glycine max;* $\mathrm{Cm^R}$	USDA; Beltsville, MD
*Bj*Δ*cyp117*	deletion strain of CYP117 derived from *B. japonicum* USDA110 parent strain; $\mathrm{Cm^R}$	This study
*Bj*Δ*cyp114*	deletion strain of CYP114 derived from *B. japonicum* USDA110 parent strain; $\mathrm{Cm^R}$	This study
*Bj*Δ$\mathit{fd_{GA}}$	deletion strain of $\mathrm{Fd_{GA}}$ derived from *B. japonicum* USDA110 parent strain; $\mathrm{Cm^R}$	This study
*Bj*Δ$\mathit{sdr_{GA}}$	deletion strain of $\mathrm{SDR_{GA}}$ derived from *B. japonicum* USDA110 parent strain; $\mathrm{Cm^R}$	This study
*Bj*Δ*cyp112*	deletion strain of CYP112 derived from *B. japonicum* USDA110 parent strain; $\mathrm{Cm^R}$	This study
Sinorhizobium fredii NGR234 (a.k.a. *Ensifer fredii* NGR234)	widely nodulating wild-type rhizobial symbiont of *Vigna unguiculata*, among others; $\mathrm{Rf^R}$	47
*Sf*Δ*cyp117*	deletion strain of CYP117 derived from *S. fredii* NGR234 parent strain; $\mathrm{Rf^R}$	This study
*Sf*Δ*cyp114*	deletion strain of CYP114 derived from *S. fredii* NGR234 parent strain; $\mathrm{Rf^R}$	This study
*Sf*Δ$\mathit{fd_{GA}}$	deletion strain of $\mathrm{Fd_{GA}}$ derived from *S. fredii* NGR234 parent strain; $\mathrm{Rf^R}$	This study
*Sf*Δ$\mathit{sdr_{GA}}$	deletion strain of $\mathrm{SDR_{GA}}$ derived from *S. fredii* NGR234 parent strain; $\mathrm{Rf^R}$	This study
*Sf*Δ*cyp112*	deletion strain of CYP112 derived from *S. fredii* NGR234 parent strain; $\mathrm{Rf^R}$	This study

Plasmids		
pCR^{TM}-Blunt II-TOPO®	Km^R; for cloning and propagation of constructs	Thermo Fisher Scientific
pCDF-Duet^{TM}-1	Sp^R; for expression in C41 *E. coli* cells	Novagen (EMD Millipore)
pstb-LAFR5	pLAFR5 with the SMb21651 promoter sequence; Broad-range-host cosmid expression vector shown to be functional in *S. meliloti* 1021; mob+; Tc^R	50
pK19*mobsacB*	Suicide vector containing the sacB gene for negative selection; mob+; Km^R; for double cross-over homologous recombination deletion of bacterial genes	23
Expression Constructs		
pstb-LAFR5-SfCYP117	For expression of SfCYP117 in *S. meliloti* 1021; Tc^R	This study
pstb-LAFR5-SfCYP117-Fd	For coexpression of SfCYP117 and SfFd$_{GA}$ in *S. meliloti* 1021; Tc^R	This study
pstb-LAFR5-SfCYP114	For expression of SfCYP114 in *S. meliloti* 1021; Tc^R	This study
pstb-LAFR5-SfCYP114-Fd	For coexpression of SfCYP114 and SfFd$_{GA}$ in *S. meliloti* 1021; Tc^R	This study
pstb-LAFR5-SfSDR	For expression of SfSDR$_{GA}$ in *S. meliloti* 1021; Tc^R	This study
pstb-LAFR5-SfCYP112	For expression of SfCYP112 in *S. meliloti* 1021; Tc^R	This study
pstb-LAFR5-SfCYP112-Fd	For coexpression of SfCYP112 and SfFd$_{GA}$ in *S. meliloti* 1021; Tc^R	This study
pET101-BjCYP114	For expression of BjCYP114 in BL21 *E. coli* cells; Cb^R	This study
pCDF-BjSDR/Fd	For expression of BjSDR$_{GA}$ in C41 *E. coli* cells; Sp^R	This study
pCDF-BjFdR1/Fd	For coexpression of *B. japonicum* USDA110 Fd$_{GA}$ with FdR1 in BL21 *E. coli* cells; Sp^R	This study

pCDF-BjFdR2/Fd	For coexpression of *B. japonicum* USDA110 Fd_{GA} with FdR2 in BL21 *E. coli* cells; Sp^R	This study
pCDF-BjFdR3/Fd	For coexpression of *B. japonicum* USDA110 Fd_{GA} with FdR3 in BL21 *E. coli* cells; Sp^R	This study
pCDF-BjFdR4/Fd	For coexpression of *B. japonicum* USDA110 Fd_{GA} with FdR4 in BL21 *E. coli* cells; Sp^R	This study
pCDF-BjFdR5/Fd	For coexpression of *B. japonicum* USDA110 Fd_{GA} with FdR5 in BL21 *E. coli* cells; Sp^R	This study
Deletion constructs		
pK19*mobsacB*-BjCYP117-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of BjCYP117 for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-BjCYP114-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of BjCYP114 for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-$BjSDR_{GA}$-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of $BjSDR_{GA}$ for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-$BjFd_{GA}$-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of $BjFd_{GA}$ for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-BjCYP112-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of BjCYP112 for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-SfCYP117-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of SfCYP117 for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-SfCYP114-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of SfCYP114 for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-$SfSDR_{GA}$-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of $SfSDR_{GA}$ for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-$SfFd_{GA}$-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of $SfFd_{GA}$ for clean deletion of the gene through double homologous recombination	This study
pK19*mobsacB*-SfCYP112-AB/CD	Construct containing a fused DNA fragment of regions upstream (AB) and downstream (CD) of SfCYP112 for clean deletion of the gene through double homologous recombination	This study

Supplementary Table 8. Primers used to create expression constructs and deletion bacteroid strains. Bj=*Bradyrhizobium japonicum*; Sf=*Sinorhizobium fredii*. Red=restriction site within primer for cloning. **Bold**=overlap region for fusing together DNA fragments.

Name	Sequence (5' to 3')	Description
Expression constructs		
Sf-sCYP117/sCYP112-F	CGCGGATCCTAACGTAACGTAAATGGAGTCACCTCT	Forward primer to amplify sCYP117/sCYP112 and sCYP117-Fd/sCYP112-Fd; anneals to 5' linker and BamHI which are located in both synthetic constructs, allowing for use of this single primer
Sf-Fd$_{GA}$-R	CCGGAATTCTCAAACCGTTCCATCGTGTCCTCCTGG	Reverse primer to amplify sCYP117-Fd, CYP114-Fd, and sCYP112-Fd; contains EcoRI
Sf-sCYP117-R	CCGGAATTCTCATGAGAATCCGATGCGGATTGTCAT	Reverse primer to amplify sCYP117; contains EcoRI
Sf-CYP114-ORF-F	CACCATGGACATGCAAGAAACCACGACA	Forward primer to amplify the ORF of CYP114 and combined CYP114-Fd amplicon from gDNA; contains CACC for use in pENTR
Sf-CYP114-ORF-R	CTACGGTTGCGCCCTGCGATCAGTCG	Reverse primer to amplify the ORF of CYP114
Sf-CYP114-ORF-linker-F	CGCGGATCCTAACGTAACGTAAATGGAGTCACCTCTATGGACATGCAAGAAACCACGACAGC	Forward primer to add BamHI and the 5' linker sequence to the CYP114 construct
Sf-CYP114-ORF-EcoRI-R	CCGGAATTCCTACGGTTGCGCCCTGCGATCAGTCG	Reverse primer to add EcoRI onto the 3' end of the CYP114 construct
Sf-sCYP112-R	CCGGAATTCTCACCAGAGCACCGGGAACTCCTCGA	Reverse primer to amplify sCYP112; contains EcoRI
Sf-SDR-ORF-F	ATGGAACGGTTTGAAGGCAAGGTGGCCG	Forward primer to amplify ORF of SDR from gDNA
Sf-SDR-ORF-R	TCAGCAGCGCGCCGTTTCGGCGGTGTC	Reverse primer to amplify ORF of SDR from gDNA
Sf-SDR-linker-F1	AATGGAGTCACCTCTATGGAACGGTTTGAAGGCAAGGTGG	Forward primer to add 15 bp of linker sequence to the 5' end of SDR construct
Sf-SDR-EcoRI-R	CCGGAATTCTCAGCAGCGCGCCGTTTCGGCG	Reverse primer to add EcoRI onto the 3' end of the SDR construct
Sf-SDR-linker-F2	CGCGGATCCTAACGTAACGTAAATGGAGTCACCTCT	Forward primer used to add the remainder of the linker sequence and a 5' BamHI site to the SDR construct
Knockout constructs		
Bj-CYP117ko-A-F	CATGGAATTCAACCGGACGGCGTGGCCGAAGTGTG	Forward primer to amplify a ~1kb region upstream of CYP117 (AB fragment); contains 5' EcoRI
Bj-CYP117ko-B-R	**CACACGGGGCATGCGATGCCAGC**CAGCAGCGCGGCGCCGCGTCCGCTTC	Reverse primer to amplify ~1kb region upstream of CYP117 (AB fragment); contains overlap region for annealing/fusing to CD fragment
Bj-CYP117ko-C-F	**GCGGACGCGGCGCCGCGCTGCTG**GCTGGCATCGCATGCCCCGTGTGGTG	Forward primer to amplify a ~1kb region downstream of CYP117 (CD fragment); contains overlap region for annealing/fusing to AB fragment
Bj-CYP117ko-D-R	CATGAAGCTTTCATGCGCGTGGCGCGTGTTTGAAC	Reverse primer to amplify ~1kb region downstream of CYP117 (CD fragment); contains HindIII
Bj-CYP117ko-check-F	ATCGTCAACATGTCGTCGTGCCAGG	Forward primer ~400bp upstream to check for presence or deletion of CYP117; wild-type fragment=2.1 kb, knockout=0.8 kb

Bj-CYP117ko-check-R	TGCGCCGGCAGCCAAACAGAGCAAG	Reverse primer ~400 bp downstream to check for presence or deletion of CYP117; wild-type fragment=2.1 kb, knockout=0.8 kb
Bj-CYP114ko-A-F	GCGAATTCGTCCCTGGTCGACAAGGCGGTAGAG	Forward primer to amplify ~500bp region upstream of CYP117 (AB fragment); contains 5' EcoRI
Bj-CYP114ko-B-R	**ACGCGCATC**CCTGGTTACCTGTTGGCGGGCTG	Reverse primer to amplify ~500bp region upstream of CYP114 (AB fragment); contains overlap region for annealing/fusing to CD fragment
Bj-CYP114ko-C-F	**GTAACCAGG**GATGCGCGTCATGGTCGAC	Forward primer to amplify a ~500bp region downstream of CYP114 (CD fragment); contains overlap region for annealing/fusing to AB fragment
Bj-CYP114ko-D-R	ACTAAGCTTCTGCGCATCGGCGATGTCGAT	Reverse primer to amplify ~500bp region downstream of CYP117 (CD fragment); contains HindIII
Bj-CYP114ko-check-F	ATTCCCGCGGAGAGCAAGGTGCTGG	Forward primer ~400bp upstream to check for presence or deletion of CYP114; wild-type fragment=2.2 kb, knockout=0.8 kb
Bj-CYP114ko-check-R	ATAGCCGCCGAGCCATCAATGTCGGC	Reverse primer ~400bp downstream to check for presence or deletion of CYP114; wild-type fragment=2.2 kb, knockout=0.8 kb
Bj-Fd$_{GA}$ko-A-F	ACCGAGAATTCTCATACAGCGCAAGCGCACCGATCC	Forward primer to amplify ~600bp region upstream of Fd$_{GA}$ (AB fragment); contains 5' EcoRI
Bj-Fd$_{GA}$ko-B-R	**TGCCTTCAAACCGTCCCATC**GAACGTCCGAAGGAATGCCGAGG	Reverse primer to amplify ~600bp region upstream of Fd$_{GA}$ (AB fragment); contains overlap region for annealing/fusing to CD fragment
Bj-Fd$_{GA}$ko-C-F	**CGGCATTCCTTCGGACGTTC**GATGGGACGGTTTGAAGGCAAAGTGG	Forward primer to amplify a ~800bp region downstream of Fd$_{GA}$ (CD fragment); contains overlap region for annealing/fusing to AB fragment
Bj-Fd$_{GA}$ko-D-R	TCGGAGAATTCCTGTTGCCACCGTCGGCGTATGTCG	Reverse primer to amplify ~800bp region downstream of Fd$_{GA}$ (CD fragment); contains EcoRI
Bj-Fd$_{GA}$ko-check-F	TCGAAGAAGTCCTGCGCTGCGACGC	Forward primer ~400bp upstream to check for presence or deletion of Fd$_{GA}$; wild-type fragment=1.1 kb, knockout=0.8 kb
Bj-Fd$_{GA}$ko-check-R	ATGTTGACGATCGCGCCACCGCCGC	Reverse primer ~400bp downstream to check for presence or deletion of Fd$_{GA}$; wild-type fragment=1.1 kb, knockout=0.8 kb
Bj-SDR$_{GA}$ko-A-F	ACCGTGAATTCTATCGCCCAGCGCGACATCGAACTGG	Forward primer to amplify ~600bp region upstream of SDR$_{GA}$ (AB fragment); contains 5' EcoRI
Bj-SDR$_{GA}$ko-B-R	**CAGCGGGTTGAGCAGCATGTCCA**CGTGTCATCCTAGATTGTGCTGGTC	Reverse primer to amplify ~600bp region upstream of SDR$_{GA}$ (AB fragment); contains overlap region for annealing/fusing to CD fragment
Bj-SDR$_{GA}$ko-C-F	**CCAGCACAATCTAGGATGACACG**TGGACATGCTGCTCAACCCGCTGAACC	Forward primer to amplify a ~700bp region downstream of SDR$_{GA}$ (CD fragment); contains overlap region for annealing/fusing to AB fragment
Bj-SDR$_{GA}$ko-D-R	GAATTCTCGTCGCTGCGATCGAAGGCGCTCACC	Reverse primer to amplify ~700bp region downstream of SDR$_{GA}$ (CD fragment); contains BamHI
Bj-SDR$_{GA}$ko-check-F	TCACTTCTGCCTCGGTGCGCAACTG	Forward primer ~400bp upstream to check for presence or deletion of SDR$_{GA}$; wild-type fragment=1.7 kb, knockout=0.8 kb
Bj-SDR$_{GA}$ko-check-R	TAGATCGCCGGTTTCGCGCAGGATG	Reverse primer ~400bp downstream to check for presence or deletion of SDR$_{GA}$; wild-type fragment=1.7 kb, knockout=0.8 kb
Bj-CYP112ko-A-F	GCGGATCCGCACATTCAGCGCGAGAGCAGG	Forward primer to amplify ~500bp region upstream of CYP112 (AB fragment); contains 5' BamHI

Bj-CYP112ko-B-R	**CGCGACA**GGTGGCACTTCACTCTCTAAG	Reverse primer to amplify ~500bp region upstream of CYP112 (AB fragment); contains overlap region for annealing/fusing to CD fragment
Bj-CYP112ko-C-F	**GAAGTGCCACC**TGTCGCGGACGCCACG	Forward primer to amplify a ~500bp region downstream of CYP112 (CD fragment); contains overlap region for annealing/fusing to AB fragment
Bj-CYP112ko-D-R	ACTAAGCTTTGCCGTCCAGGCGCGCTG	Reverse primer to amplify ~500bp region downstream of CYP112 (CD fragment); contains HindIII
Bj-CYP112ko-check-F	AGCTATGTAGTGCCTGCTGAGCCTGC	Forward primer ~400bp upstream to check for presence or deletion of CYP112; wild-type fragment=2.0 kb, knockout=0.8 kb
Bj-CYP112ko-check-R	TATGCGTCGGTGGGTCCTTCATCGC	Reverse primer ~400bp downstream to check for presence or deletion of CYP112; wild-type fragment=2.0 kb, knockout=0.8 kb
Sf-CYP117ko-A-F	ATAAGGATCCCAGACCATGGCGACCAATCTGCGCG	Forward primer to amplify a ~1kb region upstream of CYP117 (AB fragment); contains 5' BamHI
Sf-CYP117ko-B-R	**GCATGCGATGCCAGC**CAGCAGCGCGCCGTTTCGGCGGTGT	Reverse primer to amplify ~1kb region upstream of CYP117 (AB fragment); contains overlap region for annealing/fusing to CD fragment
Sf-CYP117ko-C-F	**AAACGGCGCGCTGCTG**GCTGGCATCGCATGCCCCGTGTGG	Forward primer to amplify a ~1kb region downstream of CYP117 (CD fragment); contains overlap region for annealing/fusing to AB fragment
Sf-CYP117ko-D-R	TAATAAGCTTAGGTCGTCGTGGACCAGGGTACAAG	Reverse primer to amplify ~1kb region downstream of CYP117 (CD fragment); contains HindIII
Sf-CYP117ko-check-F	CGTCAACATGTCGTCGTGCCAGGG	Forward primer ~400bp upstream to check for presence or deletion of CYP117; wild-type fragment=2.1 kb, knockout=0.8 kb
Sf-CYP117ko-check-R	CCAGTGCTCCAGCAGCCAGACAGAGC	Reverse primer ~400 bp downstream to check for presence or deletion of CYP117; wild-type fragment=2.1 kb, knockout=0.8 kb
Sf-CYP114ko-A-F	ATAAGGATCCCCATCCCTGGTGGACAAGGCGGTGG	Forward primer to amplify ~400bp region upstream of CYP117 (AB fragment); contains 5' BamHI
Sf-CYP114ko-B-R	**CCTTTTCATGCGGTT**CACCAGAGCACCGGGAACTCCTCGA	Reverse primer to amplify ~400bp region upstream of CYP114 (AB fragment); contains overlap region for annealing/fusing to CD fragment
Sf-CYP114ko-C-F	**TCCCGGTGCTCTGGTG**AACCGCATGAAAAGGGTACAAGGG	Forward primer to amplify a ~500bp region downstream of CYP114 (CD fragment); contains overlap region for annealing/fusing to AB fragment
Sf-CYP114ko-D-R	TAATAAGCTTGCCAGCGCATTGCCCGCTTCGGCCG	Reverse primer to amplify ~500bp region downstream of CYP117 (CD fragment); contains HindIII
Sf-CYP114ko-check-F	GACGGCATCATGCGCTATCCGAGG	Forward primer ~400bp upstream to check for presence or deletion of CYP114; wild-type fragment=2.2 kb, knockout=0.8 kb
Sf-CYP114ko-check-R	TCAAACCGTTCCATCGTGTCCTCCTGG	Reverse primer ~400bp downstream to check for presence or deletion of CYP114; wild-type fragment=2.2 kb, knockout=0.8 kb
Sf-Fd$_{GA}$ko-A-F	CATGGAATTCACCTGCATCGGCAATTCGCGCTGGC	Forward primer to amplify ~1kb region upstream of Fd$_{GA}$ (AB fragment); contains 5' EcoRI
Sf-Fd$_{GA}$ko-B-R	**ACCTTGCCTTCAAACCGTTCCAT**CTCAGCCCTTGTACCCTTTTCATGCGG	Reverse primer to amplify ~1kb region upstream of Fd$_{GA}$ (AB fragment); contains overlap region for annealing/fusing to CD fragment
Sf-Fd$_{GA}$ko-C-F	**ATGAAAAGGGTACAAGGGCTGAG**ATGGAACGGTTTGAAGGCAAGGTGGCC	Forward primer to amplify a ~1kb region downstream of Fd$_{GA}$ (CD fragment); contains overlap region for annealing/fusing to AB fragment

Sf-Fd$_{GA}$ko-D-R	CATGAAGCTTAGTGGCTGCCCAGCGTCCGTTCCGC	Reverse primer to amplify ~1kb region downstream of Fd$_{GA}$ (CD fragment); contains HindIII
Sf-Fd$_{GA}$ko-check-F	TAGCTTCGGCCACGGCATCCACTTCTG	Forward primer ~300bp upstream to check for presence or deletion of Fd$_{GA}$; wild-type fragment=0.9 kb, knockout=0.6 kb
Sf-Fd$_{GA}$ko-check-R	CCGAAGTGCCGCTCCGCCGTCTCGAA	Reverse primer ~300bp downstream to check for presence or deletion of Fd$_{GA}$; wild-type fragment=0.9 kb, knockout=0.6 kb
Sf-SDR$_{GA}$ko-A-F	CATGGAATTCCACGCTATCGGATGCGGAGTTGATCAGC	Forward primer to amplify ~1kb region upstream of SDR$_{GA}$ (AB fragment); contains 5' EcoRI
Sf-SDR$_{GA}$ko-B-R	**TAGGGGGTTGAGCAGCACGTTCA**CGTGTCCTCCTGGAATGCGTTGGTCTTTC	Reverse primer to amplify ~1kb region upstream of SDR$_{GA}$ (AB fragment); contains overlap region for annealing/fusing to CD fragment
Sf-SDR$_{GA}$ko-C-F	**CCAACGCATTCCAGGAGGACACG**TGAACGTGCTGCTCAACCCCCTAAACCG	Forward primer to amplify a ~1kb region downstream of SDR$_{GA}$ (CD fragment); contains overlap region for annealing/fusing to AB fragment
Sf-SDR$_{GA}$ko-D-R	CATGAAGCTTACAGATGGGTGCCCGCCGGAATGCGC	Reverse primer to amplify ~1kb region downstream of SDR$_{GA}$ (CD fragment); contains HindIII
Sf-SDR$_{GA}$ko-check-F	CGTCGTGATTGACCAAGATCTGTGCGG	Forward primer ~400bp upstream to check for presence or deletion of SDR$_{GA}$; wild-type fragment=1.6 kb, knockout=0.75 kb
Sf-SDR$_{GA}$ko-check-R	CGCCGGTTTCGGGCAGGATGGTTACGT	Reverse primer ~400bp downstream to check for presence or deletion of SDR$_{GA}$; wild-type fragment=1.6 kb, knockout=0.75 kb
Sf-CYP112ko-A-F	ATAAGGATCCCCACGCACAATTCCGCAACGGTCG	Forward primer to amplify ~500bp region upstream of CYP112 (AB fragment); contains 5' BamHI
Sf-CYP112ko-B-R	**GGCGGCGTCCGCGCA**GGTGGCACTCCACTCTCTAAGCGAT	Reverse primer to amplify ~500bp region upstream of CYP112 (AB fragment); contains overlap region for annealing/fusing to CD fragment
Sf-CYP112ko-C-F	**GTGGAGTGCCACC**TGCGCGGACGCCGCCGGGAATCGC	Forward primer to amplify a ~500bp region downstream of CYP112 (CD fragment); contains overlap region for annealing/fusing to AB fragment
Sf-CYP112ko-D-R	TAATAAGCTTCATGCCGTCCAGGCGCGCTGCGACG	Reverse primer to amplify ~500bp region downstream of CYP112 (CD fragment); contains HindIII
Sf-CYP112ko-check-R	CCTGTCGTCGGCAAATTTCGTTTGAGC	Forward primer ~400bp upstream to check for presence or deletion of CYP112; wild-type fragment=2.0 kb, knockout=0.8 kb
Sf-CYP112ko-check-F	GCAGACGCGTATGCGTCGGTGGTTCC	Reverse primer ~400bp downstream to check for presence or deletion of CYP112; wild-type fragment=2.0 kb, knockout=0.8 kb
sacB-F	GTGCGTAACTAACTTGCCATCTTC	Forward primer to amplify a portion of the sacB gene for confirmation of pK19*mobsacB* knockout construct integration into the host genome
sacB-R	CCGAAGCCCAACCTTTCATAGAAG	Reverse primer to amplify a portion of the sacB gene for confirmation of pK19*mobsacB* knockout construct integration into the host genome

Supplementary Table 9. List of genes characterized in this study. Provided are the NCBI/GenBank gene symbols, gene description, gene ID's, locus tags, and UniProtKB identifiers.

	gene symbol	**gene description**	**gene ID**	**locus tag**	**UniProtKB identifier**
***Bradyrhizobium japonicum* USDA110**					
CYP112	cyp112	cytochrome P-450 BJ-1	1055403	blr2144	Q59203 (CPXP_BRADU)
CYP114	cyp114	cytochrome P-450 BJ-3	1055406	blr2145	Q59204 (CPXR_BRADU)
$\mathrm{Fd}_{\mathrm{GA}}$	not annotated	P450-system 3Fe-4S ferredoxin	-	blr2145.1	Q45218 (FE45_BRADU)
$\mathrm{SDR}_{\mathrm{GA}}$	blr2146	dehydrogenase	1055404	blr2146	Q45219 (Y2146_BRADU)
CYP117	cyp117	cytochrome P-450 BJ-4	1055402	blr2147	Q59205 (CPXU_BRADU)
***Sinorhizobium fredii* NGR234**					
CYP112	cpxP	cytochrome P450 protein CpxP	962601	NGR_a02700	P55544 (CPXP_RHISN)
CYP114	cpxR	cytochrome P450 protein CpxR	962600	NGR_a02710	P55543 (CPXR_RHISN)
$\mathrm{Fd}_{\mathrm{GA}}$	NGR_a02720	P450-system 3Fe-4S ferredoxin protein	962355	NGR_a02720	P55542 (FE45_RHISN)
$\mathrm{SDR}_{\mathrm{GA}}$	NGR_a02730	short chain dehydrogenase/reductase	962611	NGR_a02730	P55541 (Y4LA_RHISN)
CYP117	cpxU	cytochrome P450	962613	NGR_a02740	P55540 (CPXU_RHISN)