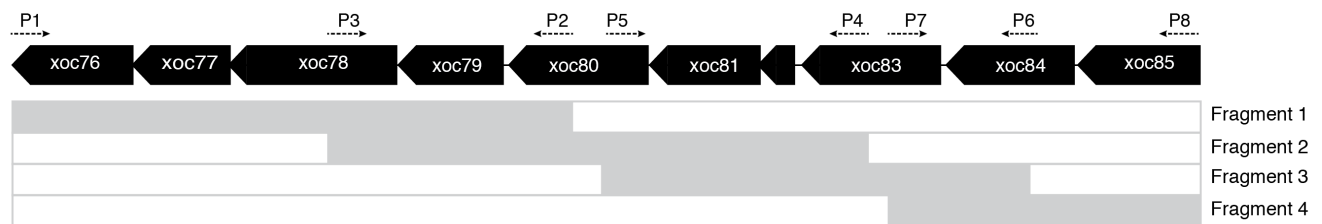


Supporting Information for:

An operon for production of bioactive gibberellin A₄ phytohormone with wide distribution in the bacterial rice leaf streak pathogen *Xanthomonas oryzae* pv. *oryzicola*

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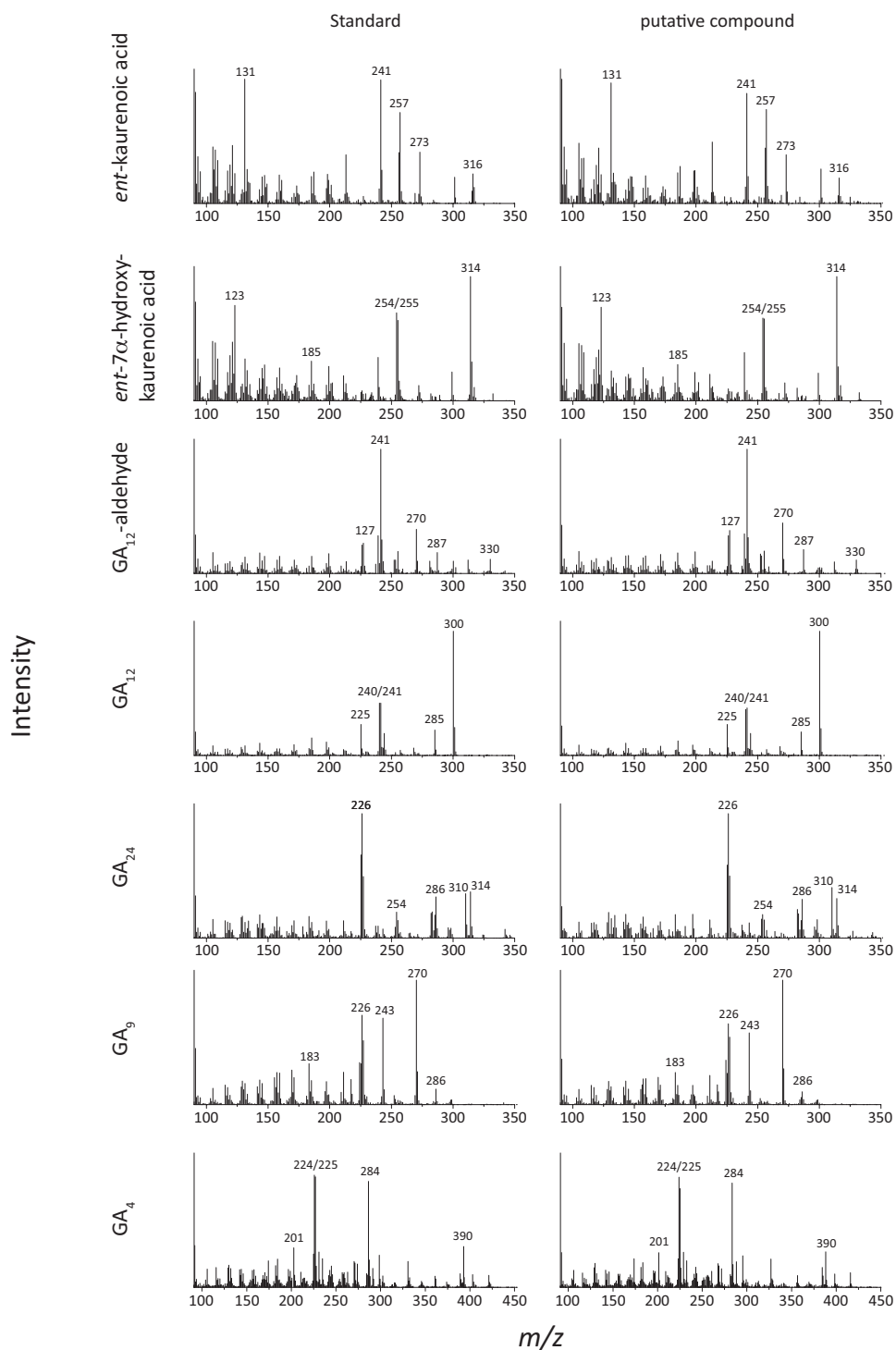
Article acceptance date: 10 December 2016



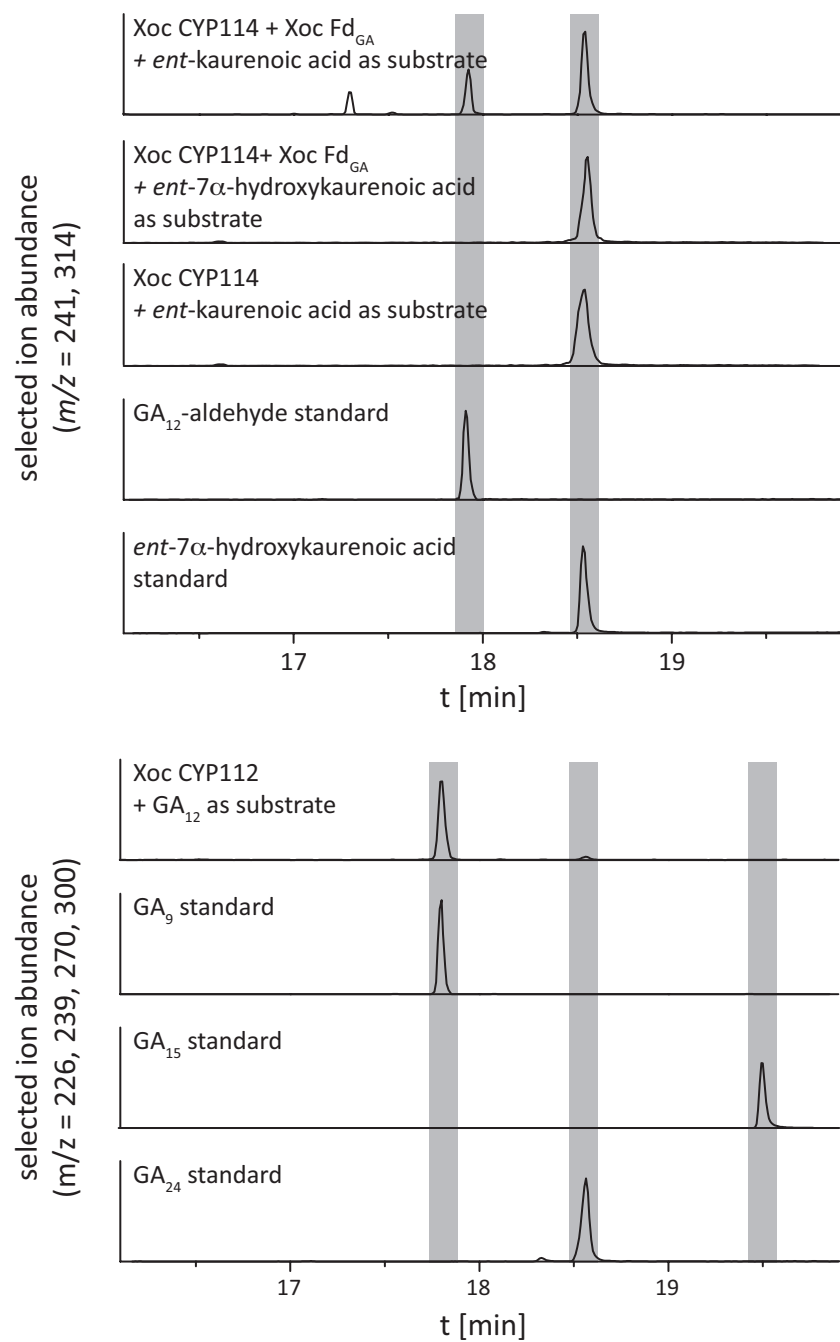
Supporting Figure S1: Strategy used for PCR screening of Xoc isolates. Genes comprising the GA biosynthetic operon are indicated in black blocks. Grey bars represent overlapping amplification products. Primers are indicated as dashed arrows (primer sequence can be found in Table S1).

Supporting Table S1: Primers used in this study

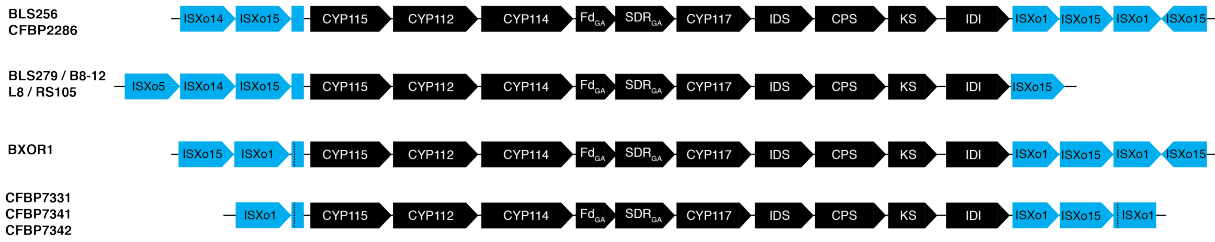
Primer ID	Primer sequence (5' to 3')		Product size (bp)
	Forward	Reverse	
<i>Cloning Xoc genes for heterologous expression</i>			
Xoc CYP117	CACCATGGACGTGCTGCGCAACCC	TCATGCGAAGCCGATATGGACGG	
Xoc CYP114	CACCATGGCCGTACAAGCAGCACACAG	TCAGCCATCGTGCACCGCC	
Xoc Fd _{GA}		TCACATCGTCCCATCGTGGCC	
Xoc SDR _{GA}	CACCATGGGACGATGTGAAGGCAAAGTGG	TCAGCGATCCACCCCGACG	
Xoc CYP112	CACCATGTCTAGACGATCCGTTGCCGATGC	TCACCAGCGAACC GGGAACG	
Xoc CYP115	CACCATGTGGCCTGAAAGCGATCATTGTG	CTAGACGGCAGAAGGGCGAAAGGTGATC	
<i>GA biosynthetic operon screening</i>			
Fragment 1	TCCGCGAGGTAATACCTAATCCAGTG	CCGCTGCTGCATTTCTC	4929
Fragment 2	CTGGCTCGAACACATTGATA	ACCGTACTGGGAGGATTGA	5045
Fragment 3	GCGGGGCCGAAGTCC	CGCCGCTGCCTTAGATGT	4146
Fragment 4	GCGTCCGGCGGCTCCTT	GGTCCGGGCTAATCAGTTCT	3001



Supporting Figure S2: Mass spectra of peaks from GC-chromatograms. Mass spectra of peaks shown in Figure 2 and Supplemental Figure 2 were compared to the mass spectra of the corresponding authentic standards to confirm their identity.



Supporting Figure S3: Activity of recombinant XocCyp117, Cyp114+/-Fd_{GA} and Cyp112. GC-MS chromatograms of hexane extracts from *E. coli* cultures expressing the indicated enzymes and chromatograms of standard compounds as a reference. Just as with *B. japonicum* and *S. fredii* (Nett *et al.*, 2016), *ent*-7 α -hydroxykaurenoic acid is not further transformed by cultures co-expressing CYP114 and Fd_{GA}.



Supporting Figure S4: Insertional sequence (IS) elements flanking the GA biosynthetic operon in *Xoc* the indicated isolates.

Supporting Table S2. Isolates of *X. oryzae* pv. *oryzicola* screened for GA biosynthetic operon.

<i>Xanthomonas</i> Isolate	Year	Country	Town	Province	Host	XODB fragments			
						1	2	3	4
<i>oryzae</i> pv. <i>oryzicola</i>									
MAI_03	2003	Mali	O. du Niger	Niono	<i>O. sativa</i>				
MAI_04	-	Mali	-	-	-				
MAI_05		Mali							
MAI_06	-	Mali	-	-	-				
MAI_07	2003	Mali	O. du Niger	Niono	<i>O. sativa</i> Jamajigi				
MAI_10 ^a	2003	Mali	O. du Niger	Niono	<i>O. sativa</i>				
MAI_11	2003	Mali	O. du Niger	Niono	<i>O. sativa</i>				
MAI_16	2009	Mali	O. du Niger	Molodo	<i>O. sativa</i>				
MAI_18	2009	Mali	O. du Niger	Kogoni	<i>O. longistaminata</i>				
MAI_19	2009	Mali	O. du Niger	Kogoni	<i>O. longistaminata</i>				
MAI_20	2009	Mali	O. du Niger	Kogoni	<i>O. longistaminata</i>				
MAI_21	2009	Mali	O. du Niger	Kogoni	<i>O. longistaminata</i>				
MAI_22	2009	Mali	O. du Niger	Kogoni	<i>O. barthii</i>				
MAI_23	2009	Mali	O. du Niger	Kogoni	<i>O. barthii</i>				
MAI_24	2009	Mali	O. du Niger	Kogoni	<i>O. barthii</i>				
MAI_25	2009	Mali	O. du Niger	Kogoni	<i>O. longistaminata</i>				
MAI_26	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_28	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_29	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_30	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_31	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_32	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_33	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_34	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_35	2009	Mali	O. du Niger	Macina	<i>O. sativa</i>				
MAI_36	2009	Mali	O. du Niger	Ndebougou	<i>O. barthii</i>				
MAI_37	2009	Mali	O. du Niger	Niono	<i>O. sativa</i>				
MAI_38	2009	Mali	Koulikoro	Dialakoroba	<i>O. sativa</i>				

MAI_39	2009	Mali	Sikasso	Sélingué	<i>O.sativa</i>				
MAI_40	2009	Mali	Sikasso	Sélingué	<i>O.sativa</i>				
MAI_41	2009	Mali	Sikasso	Sélingué	<i>O.sativa</i>				
MAI_42	2009	Mali	Sikasso	Sélingué	<i>O.sativa</i>				
MAI_43	2009	Mali	Sikasso	Sélingué	<i>O.sativa</i>				
MAI_44	2009	Mali	Sikasso	Sélingué	<i>O.sativa</i>				
MAI_46	2009	Mali	Koulikoro	Baguineda	<i>O.sativa</i>				
BAI_05 ^a	2009	B. Faso	HautsBassins	Vallée	<i>O.sativa</i>				
BAI_06	2009	B. Faso	HautsBassins	Vallée	<i>O.sativa</i>				
BAI_07	2009	B. Faso	EastCenter	Itenga	<i>O.longistaminata</i>				
BAI_08	2009	B. Faso	EastCenter	Itenga	<i>O.longistaminata</i>				
BAI_09	2009	B. Faso	EastCenter	Itenga	<i>O.longistaminata</i>				
BAI_10	2009	B. Faso	EastCenter	Itenga	<i>O.longistaminata</i>				
BAI_11 ^a	2009	B. Faso	EastCenter	Itenga	Wild rice				
BAI_12	2009	B. Faso	EastCenter	Bagré	<i>O.sativa</i>				
BAI_13	2009	B. Faso	EastCenter	Bagré	Weeds				
BAI_14	2009	B. Faso	EastCenter	Bagré	<i>O.sativa</i>				
BAI_15	2009	B. Faso	EastCenter	Bagré	<i>O.sativa</i>				
BAI_16	2009	B. Faso	EastCenter	Bagré	<i>O.sativa</i>				
BAI_17	2009	B. Faso	EastCenter	Bagré	<i>Pennisetum</i> sp.				
BAI_18	2009	B. Faso	EastCenter	Koulwooko	<i>O.sativa</i>				
BAI_19	2009	B. Faso	Cascades	Karfiguela	<i>O.sativa</i>				
BAI_20	2009	B. Faso	Cascades	Karfiguela	Weeds				
BAI_21	2009	B. Faso	Cascades	Karfiguela	<i>O.sativa</i>				
BAI_22	2009	B. Faso	Cascades	Karfiguela	<i>O.longistaminata</i>				
BAI_34	2009	B. Faso	Cascades	Karfiguela	<i>O.sativa</i>				
BAI_35	2010	B. Faso	Center	Nabadougou	<i>O.longistaminata</i>				
BAI_36	2010	B. Faso	B.Mouhoun	Niassan	<i>O.sativa</i>				
BAI_37	2010	B. Faso	EastCenter	Bagré	<i>O.sativa</i>				
BAI_38	2009	B. Faso	Cascades	Karfiguela	<i>O.sativa</i>				
BAI_39	2009	B. Faso	Cascades	Karfiguela	<i>O.sativa</i>				
BAI_40	2010	B. Faso	EastCenter	Bagré	<i>O.sativa</i>				
BAI_41	2010	B. Faso	Center	Nabadougou	<i>O.longistaminata</i>				
BAI_42	2010	B. Faso	EastCenter	Bagré	<i>O.sativa</i>				
BAI_43	2010	B. Faso	HautsBassins	Nienadonkele	<i>O.sativa</i>				
BAI_44	2010	B. Faso	HautsBassins	Nienadonkele	<i>O.sativa</i>				
BAI_49	2011	B. Faso	B. Mouhon	Niassan	<i>O. longistaminata</i>				
BAI_60	2011	B. Faso	B. Mouhon	Niassan	<i>O. sativa</i>				
BAI_82	2011	B. Faso	Cascades	Karfiguela	<i>O. sativa</i>				
BLS_001	1978	Philippines	Laguna	College	-				
BLS_030	1978	Philippines	Laguna	College	-				
BLS_046	1980	Philippines	Nueva Ecija	MRRTC	Peta				
BLS_098	1982	Philippines	Nueva Ecija	Talavera	IR52				
BLS_099	1982	Philippines	Bacolod	Sum-Ag	-				
BLS_102	1982	Philippines	Benguet	Baguio	-				
BLS_103	1982	Philippines	Benguet	Baguio	-				
BLS_105	1982	Philippines	Laguna	College	-				

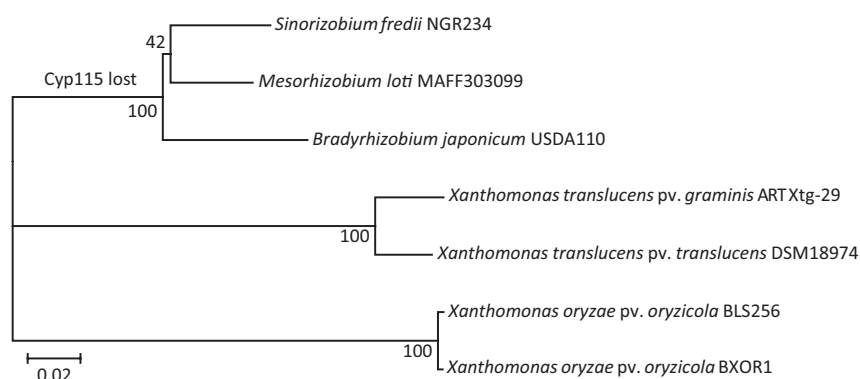
BLS_106	1982	Philippines	Camarines Sur			-				
BLS_123	1983	Philippines	Laguna	College		-				
BLS_125	1983	Philippines	Laguna	College		-				
BLS_150	1983	Philippines	Laguna	College		-				
BLS_159	1983	Philippines	Laguna	College		-				
BLS_175	1983	Philippines	North Cotabato			-				
BLS_176	1983	Philippines	North Cotabato			-				
BLS_222	1983	Philippines	Laguna	College		-				
BLS_256 ^a	1984	Philippines	IRRI-IRTP	Los Baños	IR29692-99-3-2-1					
BLS_276	1984	Philippines	Laguna	College		-				
BLS_279 ^a	1984	Philippines	Laguna	College		-				
BLS_280	1984	Philippines	Laguna	College		-				
BLS_281	1984	Philippines	IRRI	Los Baños		-				
BLS_285	1984	Philippines	Laguna	College		-				
BLS_289	1984	Philippines	Camarines Norte ^c			-				
BLS_290	1984	Philippines	Leyte ^c			-				
BLS_291	1985	Philippines	Iloilo ^c			-				
BLS_294	1984	Philippines	Nueva Ecija ^c			-				
BLS_295	1984	Philippines	North Cotabato ^c			-				
BLS_303	1985	Philippines	Iloilo	San Dionisio	C732-14(upland)					
BLS_305	1985	Philippines	Iloilo	San Dionisio	C894-21					
BLS_348	1988	Philippines	Cagayan	Alilino		-				
BLS_354 ^b	1983	Philippines	Isabela	Cabatuan		-				
BLS_356	1988	Philippines	Isabela	San Fabian	IR68					
BLS_357	1988	Philippines	Isabela	San Manuel		-				
BLS_365	1988	Philippines	Isabela	Burgos		-				
BLS_372	1989	Philippines	Nueva Ecija	CLSU, Muñoz	C4					
BLS_377	1989	Philippines	Nueva Ecija	Guimba		-				
BLS_391	1989	Philippines	Nueva Ecija	Bagsak, Calinan		-				
BLS_413	1990	Philippines	Tarlac	La Paz	O. sativa IR72					
BLS_415	1990	Philippines	Tarlac	La Paz	O. sativa IR72					
BLS_417	1990	Philippines	Tarlac	La Paz	O. sativa IR72					
BLS_420	1990	Philippines	Tarlac	La Paz	O. sativa IR72					
RS105 ^a	1992	China								
B8-12 ^a	2007	China								
L8 ^a	<	China								
Xoc China	1990	China	-	-		-				
CFBP2286 ^a	1964	Malaysia	na	na	O. sativa					
CFBP2287	-	Malaysia	-	-		-				
UPB497		Malaysia	-	-		-				
BXOR1 ^a	1996	India								
<i>oryzae</i> pv. <i>oryzae</i>										
BAI3	2003	Burkina	East center	Bagre						
MAFF311018	na	Japan								
37012	2003	Mali		Niono						
PXO113	-	Philippines								

Thai Xoo12	-	Thailand							
KACC10331 ^a		Korea							
PXO99A ^a	1980	Philippines							
PXO86 ^a	1977	Philippines							
PXO282 ^a	1990	Philippines							
PXO83 ^a	1976	Philippines	Nueva Ecija						
AXO1947 ^a	2015	Cameroon							
PXO602 ^a	2006	Philippines							
PXO71 ^a	1974	Philippines							
PXO236 ^a	1989	Philippines							
PXO145 ^a	1982	Philippines							
PXO211 ^a	1989	Philippines							
PXO524 ^a	1994	Philippines							
PXO563 ^a	1998	Philippines							
<i>campestris</i> pv. <i>leersiae</i>									
BAI23	2009	Burkina	Cascades	Karfiguela	Weeds				
<i>oryzae</i>									
US-X8-1A		USA	Lousiana		Rice				

^a Genome published

^b Genome sequenced (personal communication A. Bogdanove)

^c Town of collection unknown



Supporting Figure S5: Phylogeny of the GA biosynthetic operon. Unrooted Maximum likelihood phylogenetic tree of the concatenated amino acid sequence spanning CYP112 to KS, which includes the proteins found in all examples of the GA biosynthetic operon (the scale bar represents substitutions per site). These sequences were aligned in MEGA 7 using the Muscle algorithm, the phylogenetic tree was constructed and tested with the bootstrap method (1,000 replicate runs) in the same program using the Maximum likelihood algorithm with the JTT model and gamma distribution including all sites.

Reference:

Nett RS, Montanares M, Marcassa A, Lu X, Nagel R, Charles TC, Hedden P, Rojas MC, Peters RJ. 2016. Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution. *Nat Chem Biol*. DOI:10.1038/nchembio.2232