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An operon for production of bioactive gibberellin A_4 phytohormone with wide distribution in the bacterial rice leaf streak pathogen *Xanthomonas oryzae* pv. *oryzicola*

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Summary

- Phytopathogens have developed elaborate mechanisms to attenuate the defense response of their host plants, including convergent evolution of complex pathways for production of the gibberellin (GA) phytohormones, which were actually first isolated from the rice fungal pathogen *Gibberella fujikuroi*. The rice bacterial pathogen *Xanthomonas oryzae* pv. *oryzicola* (Xoc) has been demonstrated to contain a biosynthetic operon with cyclases capable of producing the universal GA precursor *ent*-kaurene. Genetic (knock-out) studies indicate that the derived diterpenoid serves as

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Author contributions
RN cloned the genes, performed the heterologous expression, analyzed data and wrote the manuscript, RSN aided in data analysis and writing the manuscript, RJP aided in data analysis and writing the manuscript, PT performed DNA amplifications, analyzed data and wrote the manuscript, JEL and VV provided the Xoc and Xoo DNA collection, MAVS aided in data analysis and writing the manuscript.

Supporting Information
Additional Supporting Information may be found online in the Supporting Information tab for this article:
Fig. S1 Strategy used for PCR screening of Xoc isolates.
Fig. S2 Mass spectra of peaks from GC-chromatograms.
Fig. S3 Activity of recombinant *Xoc*Cyp117, Cyp114+/-Fd_{GA} and Cyp112.
Fig. S4 Insertional sequence (IS) elements flanking the GA biosynthetic operon in Xoc.
Fig. S5 Phylogeny of the GA biosynthetic operon.
Table S1 Primers used in this study
Table S2 Isolates of *X. oryzae* pv. *oryzicola* screened for GA biosynthetic operon

a virulence factor for this rice leaf streak pathogen, serving to reduce the jasmonic acid (JA) mediated defense response.

- Here the function of the remaining genes in the Xoc operon are elucidated and the distribution of the operon in *X. oryzae* investigated in over 100 isolates.
- The Xoc operon leads to production of the bioactive GA_4, an additional step beyond production of the penultimate precursor GA_9 mediated by the homologous operons recently characterized from rhizobia. Moreover, this GA biosynthetic operon was found to be widespread in Xoc (>90%), but absent in the other major *oryzae* pathovar.
- These results indicate selective pressure for production of GA_4 in the distinct lifestyle of Xoc, and the importance of GA to both fungal and bacterial pathogens of rice.

Keywords

biogeographical distribution; cytochromes P450; gibberellin biosynthesis; plant–microbe interactions; virulence factor

Introduction

Pathogenic microbes are under intense selection to manipulate their hosts, particularly to attenuate the host defense response. An example of the complexity that can arise from this selective pressure appears to be the independent evolution of biosynthetic pathways leading to the production of plant hormones by phytopathogens. Indeed, the gibberellins (GAs), production of which requires over ten transformations from the general diterpenoid precursor (*E,E,E*)-geranylgeranyl diphosphate (GGPP), were first isolated from the rice fungal pathogen *Gibberella fujikuroi* (anamorph *Fusarium fujikuroi*) (Hedden & Sponsel, 2015). The production of GAs by *G. fujikuroi* leads to the characteristic excessive growth phenotype of the resulting bakanae or foolish seedling disease, and has recently been demonstrated to act as virulence factor for this phytopathogen (Wiemann *et al.*, 2013).

It has long been recognized that certain bacteria also produce GAs (Bottini *et al.*, 2004). Such biosynthetic capacity has been speculatively ascribed to a cytochrome P450 (CYP) rich operon widely distributed in rhizobia (Fig. 1) (Tully *et al.*, 1998; Keister *et al.*, 1999), which has been demonstrated by characterization of the encoded enzymes. The three associated synthases/cyclases lead to production of *ent*-kaurene, one is a GGPP synthase (GGPS), the next is an *ent*-copalyl diphosphate synthase (CPS), while the last is an *ent*-kaurene synthase (KS) (Morrone *et al.*, 2009; Hershey *et al.*, 2014). The three CYPs (CYP112, CYP114, and CYP117), along with the ferredoxin (Fd_{GA}) and short-chain alcohol dehydrogenase (SDR_{GA}), have recently been shown to transform *ent*-kaurene to GA_9 (although this is not thought to exhibit hormonal activity – i.e., be bioactive) in several of the rhizobia in which the operon is found (Nett *et al.*, 2016; Tatsukami & Ueda, 2016).

In addition to appearing in a sub-set of symbiotic rhizobia from the *Rhizobiales* order in the *Alphaproteobacteria* class (Hershey *et al.*, 2014), a similar operon also can be found in a few phytopathogens from the separate *Gammaproteobacteria* class, indicating that this has been more widely distributed by horizontal gene transfer. It has been suggested that the rice bacterial leaf streak pathogen *Xanthomonas oryzae* pv. *oryzicola* (Xoc), which contains a copy of this operon, might produce GA(s) as a virulence factor. However, only the capacity to produce *ent*-kaurene from GGPP, by functional characterization of the associated CPS and KS, has been reported to-date. Intriguingly, knocking out the CPS, KS, or CYP112 was sufficient to attenuate the virulence of Xoc, allowing rice to mount a more robust jasmonic acid (JA) mediated defense response (Lu *et al.*, 2015). Nevertheless, the fact that this operon was only found in the single *oryzicola* pathovar that had been sequenced at that time, and not in any of the several genome sequences available for other *X. oryzae* pathovars, left the broader importance of this operon and resulting *ent*-kaurene derived diterpenoid in these rice bacterial pathogens, and even Xoc more specifically, in question.

Here biochemical characterization is reported demonstrating that the enzymes encoded by this operon in Xoc can function to produce the bioactive GA_4. This represents further transformation of the GA_9 produced by the previously characterized rhizobial operon, with the relevant activity of 3β-hydroxylation carried out by the additional CYP (CYP115) found in the Xoc operon (Fig. 1). Moreover, although seemingly restricted to the *oryzicola* pathovar in *X. oryzae*, investigation of the biogeographical distribution of the GA biosynthetic operon with over 100 isolates of Xoc found widespread conservation that would be consistent with an important role for production of GA_4 specifically in this rice bacterial leaf streak pathogen.

Materials and Methods

Unless otherwise specified, chemicals were purchased from Sigma and Fisher Scientific. GA_{12}-aldehyde, GA_{12}, GA_9 and GA_4 were purchased from OlChemIm Ltd., while *ent*-7α-hydroxykaurenoic acid, GA_{15} and GA_{24} were kindly provided by Dr Peter Hedden (Rothamsted Research).

Genes were cloned by amplification from genomic DNA of Xoc strain BLS256 using Q5 Hot Start High-Fidelity DNA polymerase (NEB) according to the product manual, and using 5 μl of the high GC-content enhancer and gene specific primers (Supporting Information Table S1). The forward primer included a CACC sequence before the start codon to allow cloning via directional TOPO isomerization into pET100 (Invitrogen) according to the instructions supplied by the manufacturer. Clones were sequenced to verify the correct DNA sequence. CYP114 was cloned either alone or in tandem with the neighboring ferredoxin, using the forward primer for CYP114 and the reverse primer for Fd_{GA}.

For recombinant *in vivo* feeding studies the pET100 based expression constructs for CYP117, CYP112, CYP114, CYP114+Fd_{GA}, CYP115 and SDR_{GA} were transformed into *Escherichia coli* expression strain BL21-Star (Invitrogen). Three individual colonies were inoculated into 10 ml TB broth (12 g l^{-1} casein, 24 g l^{-1} yeast extract, 0.4% glycerol), including 50 μg ml^{-1} carbenicillin, and grown at 18°C for 2 d with constant shaking at 200 rpm. From these starter cultures, 2 ml were transferred into 25 ml fresh TB broth, including 50 μg ml^{-1} carbenicillin. Cultures expressing CYPs were induced at an OD of 0.8, while that for SDR_{GA} was induced at an OD 0.6, with 1 mM IPTG. Upon induction, 5 ml 1 M phosphate buffer pH 7.5, 1 mM aminolevulenic acid, 1 mM riboflavin, 0.1 mM $FeCl_3$ and 10 μM of specific substrates dissolved in a 1 : 1 mixture of DMSO and methanol (v : v) were added. Cultures were then grown at 18°C with constant shaking at 200 rpm for 3 d.

To analyze recombinant enzymatic activity, the fed cultures were acidified to pH 3.0 with 5 N HCl to neutralize the expected carboxylated products, then extracted three times with 50 ml hexanes (CYP117) or 50 ml ethyl acetate (CYP112, CYP114, CYP114+$\mathrm{Fd}_{\mathrm{GA}}$, CYP115 and $\mathrm{SDR}_{\mathrm{GA}}$). The organic phases were combined in a 250 ml round bottom flask and evaporated in a rotary evaporator to dryness. The flask contents were washed three times with 2 ml hexanes or ethyl acetate, with the exception of CYP117, reduced to *c.* 0.5 ml volume and purified using 1 ml silica gel in a glass Pasteur pipette, with elution via a stepwise gradient starting at 100% hexane and ending at 100% ethyl acetate, with 25% increments between solvent combinations. Fractions were methylated using diazomethane, and for reactions with CYP115, silylated with BSTFA+TMCS (99:1). Derivatized samples were dried under a stream of nitrogen, dissolved in n-hexane and analyzed by GC-MS, carried out with a 3900 Saturn GC (Varian), equipped with an HP-5MS column and coupled to a Saturn 2100T ion trap mass spectrometer detector (Varian). The injector of the GC was kept at a temperature of 250°C, the temperature gradient of the column started at 50°C, held for 3 min, which was then raised by 15°C min^{-1} to 300°C, again held for 3 min.

Bioinformatic searches for copies of the GA biosynthetic operon in *X. oryzae* were carried by BLAST searches of this species at NCBI and JGI using the characteristic *cps*, *ks* and *cyp112* genes as query sequences. Insertion sequences elements in the GA operon vicinity were first found using ISfinder (Siguier *et al.*, 2006), and then more completely elucidated by manual curation.

For screening, previously described West African (Wonni *et al.*, 2014) and Asian (Raymundo *et al.*, 1999) *X. oryzae* strains were obtained from a collection maintained at Colorado State University (Fort Collins, USA). The presence of the GA biosynthetic operon was directly investigated by PCR amplification using four overlapping fragments that together cover the entire operon (Fig. S1). Primers were designed to anneal within the genes. Fragments were amplified using Phusion High-Fidelity DNA polymerase (Thermo Scientific), according to the manufactures' instructions, in reactions optimized for GC-rich templates by the addition of 3% DMSO. Amplified fragments were detected via gel electrophoresis, using 0.8% TAE gels and GelRed stain (Biotium Inc.). In most cases all four fragments could be detected, although for six isolates only three fragments (and in one case only one), were observed. Given the extensive overlap and difficulty in amplification (presumably due to the high GC content), it was assumed that detection of any fragment indicated the presence of the operon. Nucleotide polymorphisms in the primer target sequences also may result in failure of amplification.

Results

The CPS and KS from the Xoc operon together produce *ent*-kaurene from GGPP (Lu *et al.*, 2015). Here we report functional characterization of the remaining oxidative enzymes from the operon (i.e., CYP112, CYP114, CYP115, CYP117, $\mathrm{SDR}_{\mathrm{GA}}$ and $\mathrm{Fd}_{\mathrm{GA}}$; see Fig. 1). For this purpose, each of the CYPs and the $\mathrm{SDR}_{\mathrm{GA}}$ were individually expressed in *E. coli*.

CYP114 was also co-expressed with Fd_{GA}, which has been shown to be required for full activity of this CYP, while the other CYPs presumably are efficiently reduced by endogenous Fd (Nett *et al.*, 2016). The resulting recombinant strains were fed each biosynthetic intermediate/precursor, in parallel cultures that were then extracted for analysis by gas-chromatography mass-spectrometry (GC-MS) to determine what (if any) enzymatic transformations had been catalyzed.

The results of these studies demonstrated that the enzymes in common between the Xoc operon and those from rhizobia (Fig. 1) catalyze the same reactions, such that the pathway from *ent*-kaurene to GA_9 elucidated by these studies is analogous to that recently reported for the rhizobial species *Bradyrhizobium japonicum* and *Sinorhizobium fredii* (Nett *et al.*, 2016). Briefly, CYP117 catalyzed the three oxidative transformations required to transform *ent*-kaurene to *ent*-kaurenoic acid (Fig. 2). This was further converted to GA_{12}-aldehyde by CYP114, which includes ring contraction from the 6-6-6-5 kaurane backbone to the 6-5-6-5 ring structure characteristic of the gibberellanes (Figs 2, S2). We predict that this transformation proceeds via initial conversion of *ent*-kaurenoic acid to *ent*-7α-hydroxykaurenoic acid, with the subsequent oxidative ring contraction reaction dependent on co-expression of Fd_{GA}, because cultures expressing CYP114 alone only produced *ent*-7α-hydroxykaurenoic acid (Fig. S3). SDR_{GA} efficiently catalyzes the further oxidation of GA_{12}-aldehyde to GA_{12} (Figs 2, S2). CYP112 then catalyzes the conversion of GA_{12} to GA_9, which also requires three reactions, first hydroxylation to the C-20 alcohol derivative GA_{15}, then oxidation to the aldehyde GA_{24}, before catalysis of the coupled loss of C-20 with formation of a γ-lactone ring (Figs 2, S2).

Notably, the operon in Xoc contains an additional CYP, CYP115, which is not generally present in rhizobia (Lu *et al.*, 2015). CYP115 proved to catalyze 3β-hydroxylation of GA_9 to form GA_4 (Figs 2, S2). Thus, beyond the penultimate precursor GA_9 observed with rhizobia (Mendez *et al.*, 2014), the operon in Xoc can not only be confidently assigned to GA biosynthesis, but also to further lead to production of the bioactive GA_4.

Bioinformatic searches of the sequence information currently available for *X. oryzae* indicated that the GA biosynthetic operon is present in Xoc, but not those for the closely related and other major pathovar *X. oryzae* pv. *oryzae* (Xoo), whose genomes have been fully sequenced (Lee *et al.*, 2005; Ochiai *et al.*, 2005; Salzberg *et al.*, 2008; Booher *et al.*, 2015; Quibod *et al.*, 2016). In Xoc, examination of the available genome sequences (Bogdanove *et al.*, 2011; Wilkins *et al.*, 2015) revealed that the GA biosynthetic operon is flanked by multiple insertion sequence (IS) elements. Although there is some difference in the exact composition of these, all Xoc isolates have at least one IS element on each side of the operon (Fig. S4).

The presence of mobile elements flanking the GA biosynthetic operon denotes potential mobility, and raises questions about the ephemerality of GA_4 production in Xoc. Thus, it seemed worth investigating how widespread the GA biosynthetic operon is within Xoc. For this purpose, 116 isolates from different regions where the resulting bacterial leaf streak disease is of agronomic concern, both West Africa and Asia, were screened by PCR for the presence of the GA biosynthetic operon. Included among these are ten isolates whose complete genome sequences are currently available (Wilkins *et al.*, 2015). The isolates from West Africa were collected from rice and wild grasses showing disease symptoms between 2003 and 2011 in Mali and Burkina Faso (35 and 32 isolates, respectively), while those from Asia were collected from rice only, with 41 isolates from the Philippines, collected between 1978 and 1990, along with three from Malaysia, four from China and one from India (Tables 1, S2).

Interestingly, the GA biosynthetic operon is highly prevalent in Xoc, as it is present in 106 of 116 isolates screened (91.3%), being absent in only 10 isolates (Table S2). On a regional level, the operon is absent only in a single isolate from the Philippines, and is absent somewhat more frequently in isolates from West Africa (9 of 67; 13.4%) (Fig. 3). While the isolates that do not contain the GA biosynthetic operon were all found on *Oryza sativa* rather than other species from the *Oryza* genus, this may not be significant, as only a limited number of isolates from these wild grasses were tested. To further test the generality of the observation that the GA biosynthetic operon is not present in the 13 complete genomes available for Xoo, five Xoo isolates from Asia and an isolate of *X. oryzae* (no pathovar designation) from the United States were tested, and found to not contain the operon (Table S2).

Discussion

The biochemical results reported here demonstrate that the GA biosynthetic operon in Xoc leads to the production of bioactive GA_4 (Fig. 2). This is consistent with the previous demonstration that the operon acts to produce a virulence factor that reduces the JA-mediated defense response (Lu *et al.*, 2015), as an antagonistic relationship between GA and JA has already been established (Robert-Seilaniantz *et al.*, 2007; Bari & Jones, 2009; Wasternack & Hause, 2013). The importance of such production of GA_4 for Xoc is indicated by the very high prevalence of the GA biosynthetic operon in this pathovar over both a broad geographical range and different periods of time also shown here (Fig. 3; Table S2).

While the GA biosynthetic operon is somewhat less prevalent in the West African isolates tested here, it is still widely found in this region (>85%). Interestingly, the virulence factor AvrRxo1, which (along with its chaperone Arc1) is similarly flanked by several IS elements (Zhao *et al.*, 2004; Liu *et al.*, 2014), also is more prevalent in Asian than African isolates of *X. oryzae* (Triplett *et al.*, 2016). Nevertheless, particularly given the lack of a clear pattern in more specific location or date of collection of these isolates, any underlying rationale for the slightly decreased prevalence of the GA biosynthetic operon in this region remains uncertain.

More critically, it also is unclear why the GA biosynthetic operon seems to be tightly restricted to Xoc and is not found in Xoo. This is especially puzzling given that exogenous application of bioactive GA_3 has been reported to increase the susceptibility of rice to Xoo (Qin *et al.*, 2013). Nevertheless, the selective distribution of the operon in *X. oryzae* presumably reflects a differential ability of GA_4 to act as a virulence factor in the distinct lifestyles of these pathovars. Notably, Xoc nominally enters rice leaves through stomata and propagates throughout the mesophyll parenchyma causing leaf streak disease, while Xoo enters through hydathodes and propagates in the xylem causing leaf blight (Nino-Liu *et al.*, 2006). While both Xoc and Xoo also can enter through wounds, given the association of the JA response with wounding, it can be hypothesized that such damaged tissue may provide a more important route for the entry of Xoc than Xoo.

Beyond Xoc and rhizobia, the GA biosynthetic operon has only been reported to be present in various pathovars of *Xanthomonas translucens* (Fig. S5), where it includes *CYP115* (Lu *et al.*, 2015). These phytopathogens infect either forage grasses or small grain cereal crops such as wheat, barley and rye, and cause leaf streak disease. Thus, their tissue specificity and mode of infection resemble that of Xoc (Stromberg *et al.*, 1999; Wichmann *et al.*, 2013; Pesce *et al.*, 2015). Assuming that production of GA_4 also serves to increase virulence for these phytopathogens, it can be similarly hypothesized that damaged tissue may be an important route of entry for *X. translucens* as well.

Intriguingly, while not otherwise particularly closely related (Naushad *et al.*, 2015), Xoc and *X. translucens* both infect plants from a sub-family of the Poaceae/grass family, specifically the BEP clade (GPWGII, 2012). Accordingly, it can be further speculated that the selective presence of the GA biosynthetic operon in these phytopathogens is a function of the high water-repellency of their host plant leaves (Neinhuis & Barthlott, 1997), due to their vertical/upright nature and hydrophobic surface (Koch *et al.*, 2008), which limits bacterial colonization and access to stomata (Beattie, 2011). Consistent with an important role for wounding in the entry of these pathogens, outbreaks of bacterial leaf streak disease have been correlated with the aftereffects of typhoons in rice (Nino-Liu *et al.*, 2006), and with mowing in forage grass pastures (Pesce *et al.*, 2015).

Lastly, it is notable that the final 3β-hydroxylation reaction catalyzed by the additional CYP115 found in the Xoc GA biosynthetic operon differentiates this from previously characterized operons (Fig. 1), which are all from rhizobia that seem to produce only the penultimate precursor GA_9 (Mendez *et al.*, 2014; Nett *et al.*, 2016; Tatsukami & Ueda, 2016). The presence of a short fragment of *CYP115* in rhizobia (Tully *et al.*, 1998; R. S. Nett *et al.*, unpublished), suggests that this gene was lost due to some negative effect on the symbiotic interaction of these rhizobia with their host plants. Indeed, the GA biosynthetic operon is not found in all rhizobia (Hershey *et al.*, 2014), and knock-out mutants are still able to nodulate their respective host plants (Tully & Keister, 1993; Nett *et al.*, 2016; Tatsukami & Ueda, 2016). The presence of Cyp115 and, therefore, the ability to produce bioactive GA_4, may reflect the difference between the relationships of these bacteria with their host plants. As bioactive GA suppresses the JA defense response (Robert-Seilaniantz *et al.*, 2007), rhizobia presumably benefit from maintaining the health of their host legume and it can be hypothesized that these symbionts may leave the final step in production of bioactive GA to the plant in order to not compromise the ability of the host to respond to infection by pathogenic microbes. Regardless of such speculation, the results reported here highlight the use of bioactive GAs as virulence factors by both fungal and bacterial pathogens of rice (Wiemann *et al.*, 2013; Lu *et al.*, 2015), which indicates the importance of the balance between GA and JA in the defense response of this important cereal crop plant to these disparate microbial disease agents, and is consistent with previous evidence that these hormonal interactions differ between rice and other plants such as *Arabidopsis thaliana* (De Bruyne *et al.*, 2014).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Bari R, Jones JD. Role of plant hormones in plant defence responses. Plant Mol Biol. 2009; 69(4):473–488. [PubMed: 19083153]

Beattie GA. Water relations in the interaction of foliar bacterial pathogens with plants. Annu Rev Phytopathol. 2011; 49:533–555. [PubMed: 21438680]

Bogdanove AJ, Koebnik R, Lu H, Furutani A, Angiuoli SV, Patil PB, Van Sluys MA, Ryan RP, Meyer DF, Han SW, et al. Two new complete genome sequences offer insight into host and tissue specificity of plant pathogenic *Xanthomonas* spp. J Bacteriol. 2011; 193(19):5450–5464. [PubMed: 21784931]

Booher NJ, Carpenter SC, Sebra RP, Wang L, Salzberg SL, Leach JE, Bogdanove AJ. Single molecule real-time sequencing of *Xanthomonas oryzae* genomes reveals a dynamic structure and complex TAL (transcription activator-like) effector gene relationships. Microb Genom. 2015; 1(4)doi: 10.1099/mgen.000032

Bottini R, Cassan F, Piccoli P. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol. 2004; 65(5):497–503. [PubMed: 15378292]

De Bruyne L, Höfte M, De Vleesschauwer D. Connecting growth and defense: the emerging roles of brassinosteroids and gibberellins in plant innate immunity. Molecular Plant. 2014; 7(6):943–959. [PubMed: 24777987]

Grass Phylogeny Working Group II. New grass phylogeny resolves deep evolutionary relationships and discovers C_4 origins. New Phytol. 2012; 193(2):304–312. [PubMed: 22115274]

Hedden P, Sponsel V. A century of gibberellin research. J Plant Growth Regul. 2015; 34(4):740–760. [PubMed: 26523085]

Hershey DM, Lu X, Zi J, Peters RJ. Functional conservation of the capacity for *ent*-kaurene biosynthesis and an associated operon in certain rhizobia. J Bact. 2014; 196:100–106. [PubMed: 24142247]

Keister DL, Tully RE, Van Berkum P. A cytochrome P450 gene cluster in the *Rhizobiaceae*. J Gen Appl Microbiol. 1999; 45(6):301–303. [PubMed: 12501360]

Koch K, Bhushan B, Barthlott W. Diversity of structure, morphology and wetting of plant surfaces. Soft Matter. 2008; 4:1943–1963.

Lee BM, Park YJ, Park DS, Kang HW, Kim JG, Song ES, Park IC, Yoon UH, Hahn JH, Koo BS, et al. The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice. Nucleic Acids Research. 2005; 33(2):577–586. [PubMed: 15673718]

Liu H, Chang Q, Feng W, Zhang B, Wu T, Li N, Yao F, Ding X, Chu Z. Domain dissection of AvrRxo1 for suppressor, avirulence and cytotoxicity functions. PLoS ONE. 2014; 9(12):e113875. [PubMed: 25437277]

Lu X, Hershey DM, Wang L, Bogdanove AJ, Peters RJ. An *ent*-kaurene derived diterpenoid virulence factor from *Xanthomonas oryzae* pv. oryzicola. New Phytol. 2015; 406:295–302.

Mendez C, Baginsky C, Hedden P, Gong F, Caru M, Rojas MC. Gibberellin oxidase activities in *Bradyrhizobium japonicum* bacteroids. Phytochemistry. 2014; 98:101–109. [PubMed: 24378220]

Morrone D, Chambers J, Lowry L, Kim G, Anterola A, Bender K, Peters RJ. Gibberellin biosynthesis in bacteria: separate *ent*-copalyl diphosphate and *ent*-kaurene synthases in *Bradyrhizobium japonicum*. FEBS Lett. 2009; 583(2):475–480. [PubMed: 19121310]

Naushad S, Adeolu M, Wong S, Sohail M, Schellhorn HE, Gupta RS. A phylogenomic and molecular marker based taxonomic framework for the order *Xanthomonadales*: proposal to transfer the families *Algiphilaceae* and *Solimonadaceae* to the order *Nevskiales* ord. nov. and to create a new family within the order *Xanthomonadales*, the family *Rhodanobacteraceae* fam. nov., containing the genus *Rhodanobacter* and its closest relatives. Antonie Van Leeuwenhoek. 2015; 107(2):467–485. [PubMed: 25481407]

Neinhuis C, Barthlott W. Characterization and distribution of water-repellent, self-cleaning plant surfaces. Ann Bot. 1997; 79:667–677.

Nett RS, Montanares M, Marcassa A, Lu X, Nagel R, Charles TC, Hedden P, Rojas MC, Peters RJ. Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution. Nat Chem Biol. 2016; Author, if possible, please update the doi with the volume and page range. doi: 10.1038/nchembio.2232

Nino-Liu DO, Ronald PC, Bogdanove AJ. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Mol Plant Pathol. 2006; 7(5):303–324. [PubMed: 20507449]

Ochiai H, Inoue V, Takeya M, Sasaki A, Kaku H. Genome sequence of *Xanthomonas oryzae* pv. *oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. Jarq-Japan Agricultural Research Quarterly. 2005; 39(4):275–287.

Pesce C, Bolot S, Cunnac S, Portier P, Fischer-Le Saux M, Jacques M-A, Gagnevin L, Arlat M, Noël LD, Carrère S, et al. High-quality draft genome sequence of the *Xanthomonas translucens* pv. *cerealis* pathotype strain CFBP 2541. Genome Announcements. 2015; 3(1):e01574–01514. [PubMed: 25676771]

Qin X, Liu JH, Zhao WS, Chen XJ, Guo ZJ, Peng YL. Gibberellin 20-oxidase gene OsGA20ox3 regulates plant stature and disease development in rice. Mol Plant Microbe Interact. 2013; 26(2):227–239. [PubMed: 22992000]

Quibod IL, Perez-Quintero A, Booher NJ, Dossa GS, Grande G, Szurek B, Vera Cruz C, Bogdanove AJ, Oliva R. Effector diversification contributes to *Xanthomonas oryzae* pv. *oryzae* phenotypic adaptation in a semi-isolated environment. Sci Rep. 2016; 6:34137. [PubMed: 27667260]

Raymundo AK, Briones AM, Ardales EY, Perez MT, Fernandez LC, Leach JE, Mew TW, Ynalvez MA, McLaren CG, Nelson RJ. Analysis of DNA polymorphism and virulence in Philippine strains of *Xanthomonas oryzae* pv. *oryzicola*. Plant Dis. 1999; 83:434–440.

Robert-Seilaniantz A, Navarro L, Bari R, Jones JDG. Pathological hormone imbalances. Curr Opin Plant Biol. 2007; 10(4):372–379. [PubMed: 17646123]

Salzberg SL, Sommer DD, Schatz MC, Phillippy AM, Rabinowicz PD, Tsuge S, Furutani A, Ochiai H, Delcher AL, Kelley D, et al. Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99A. BMC Genomics. 2008; 9:204. [PubMed: 18452608]

Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Research. 2006; 34(Database issue):D32–36. [PubMed: 16381877]

Stromberg KD, Kinkel LL, Leonard KJ. Relationship between phyllosphere population sizes of *Xanthomonas translucens* pv. *translucens* and bacterial leaf streak severity on wheat seedlings. Phytopathology. 1999; 89(2):131–135. [PubMed: 18944786]

Tatsukami Y, Ueda M. Rhizobial gibberellin negatively regulates host nodule number. Scientific Reports. 2016; 6:27998. [PubMed: 27307029]

Triplett LR, Shidore T, Long J, Miao J, Wu S, Han Q, Zhou C, Ishihara H, Li J, Zhao B, et al. AvrRxo1 is a bifunctional type III secreted effector and toxin-antitoxin system component with homologs in diverse environmental contexts. PLoS ONE. 2016; 11(7):e0158856. [PubMed: 27391081]

Tully RE, Keister DL. Cloning and mutagenesis of a cytochrome P-450 locus from *Bradyrhizobium japonicum* that is expressed anaerobically and symbiotically. Appl Environ Microbiol. 1993; 59:4136–4142. [PubMed: 16349113]

Tully RE, van Berkum P, Lovins KW, Keister DL. Identifcation and sequencing of a cytochrome P450 gene cluster from *Bradyrhizobium japonicum*. Biochim Biophys Acta. 1998; 1398:243–255. [PubMed: 9655913]

Wasternack C, Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Annals of Botany. 2013; 111(6):1021–1058. [PubMed: 23558912]

Wichmann F, Vorholter F-J, Hersemann L, Widmer F, Blom J, Niehaus K, Reinhard S, Conradin C, Kolliker R. The noncanonical type III secretion system of *Xanthomonas translucens* pv. *graminis* is essential for forage grass infection. Mol Plant Pathol. 2013; 14(6):576–588. [PubMed: 23578314]

Wiemann P, Sieber CM, von Bargen KW, Studt L, Niehaus EM, Espino JJ, Huss K, Michielse CB, Albermann S, Wagner D, et al. Deciphering the cryptic genome: genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveal complex regulation of secondary metabolism and novel metabolites. PLoS Pathog. 2013; 9(6):e1003475. [PubMed: 23825955]

Wilkins KE, Booher NJ, Wang L, Bogdanove AJ. TAL effectors and activation of predicted host targets distinguish Asian from African strains of the rice pathogen *Xanthomonas oryzae* pv. *oryzicola* while strict conservation suggests universal importance of five TAL effectors. Front Plant Sci. 2015; 6:536. [PubMed: 26257749]

Wonni I, Cottyn B, Detemmerman L, Dao S, Ouedraogo L, Sarra S, Tekete C, Poussier S, Corral R, Triplett L, et al. Analysis of *Xanthomonas oryzae* pv. *oryzicola* population in Mali and Burkina Faso reveals a high level of genetic and pathogenic diversity. Phytopathology. 2014; 104:520–531. [PubMed: 24199713]

Zhao B, Ardales EY, Raymundo A, Bai J, Trick HN, Leach JE, Hulbert SH. The *avrRxo1* gene from the rice pathogen *Xanthomonas oryzae* pv. *oryzicola* confers a nonhost defense reaction on maize with resistance gene *Rxo1*. Mol Plant Microbe Interact. 2004; 17(7):771–779. [PubMed: 15242171]

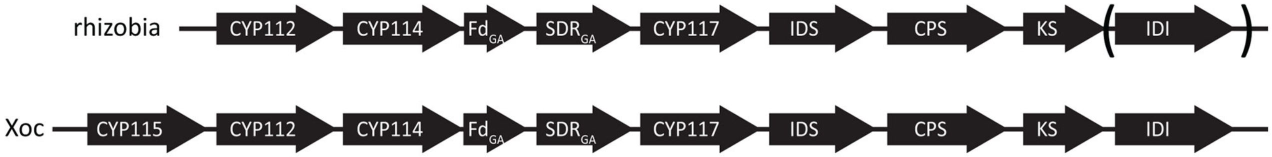

Fig. 1.

Schematic representation of the gibberellin (GA) biosynthetic operon. Abbreviations for the genes in the arrows are: CYP, cytochrome P450; Fd, ferredoxin; SDR, short-chain alcohol dehydrogenase/reductase; IDS, isoprenyl diphosphate synthase; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; and isopentenyl diphosphate isomerase (IDI), which is not found in all copies of the operon in rhizobia; the arrow indicates the direction of transcription.

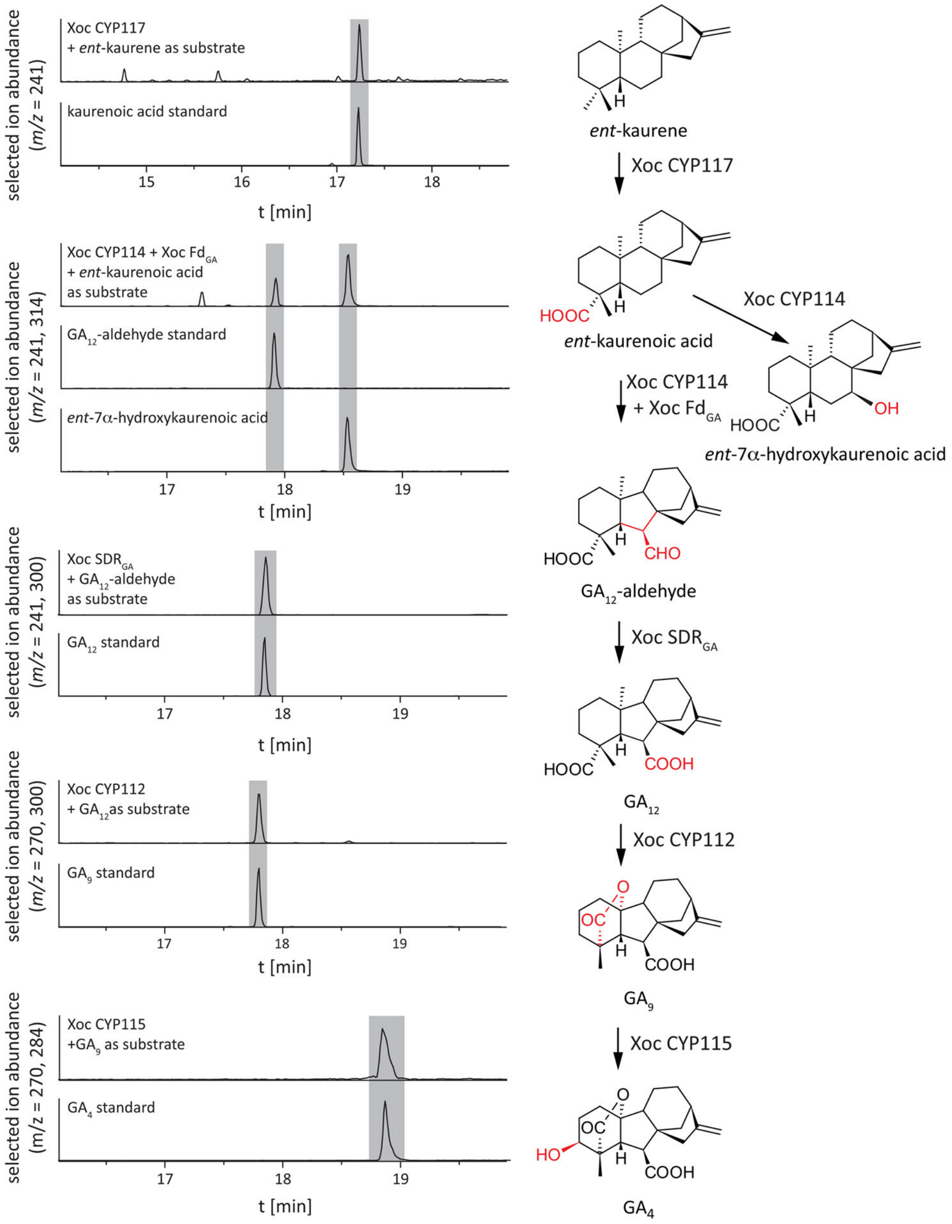

Fig. 2.

GC-MS of *Xoc*CYP117, CYP114+Fd$_{\mathrm{GA}}$, SDR$_{\mathrm{GA}}$, CYP112 and CYP115 from *Escherichia coli* extracts. (Left panel) GC-MS chromatograms of hexane extracts from *E. coli* cultures expressing the indicated enzymes and chromatograms of standard compounds as a reference. (Right panel) Reactions catalyzed by the respective enzymes.

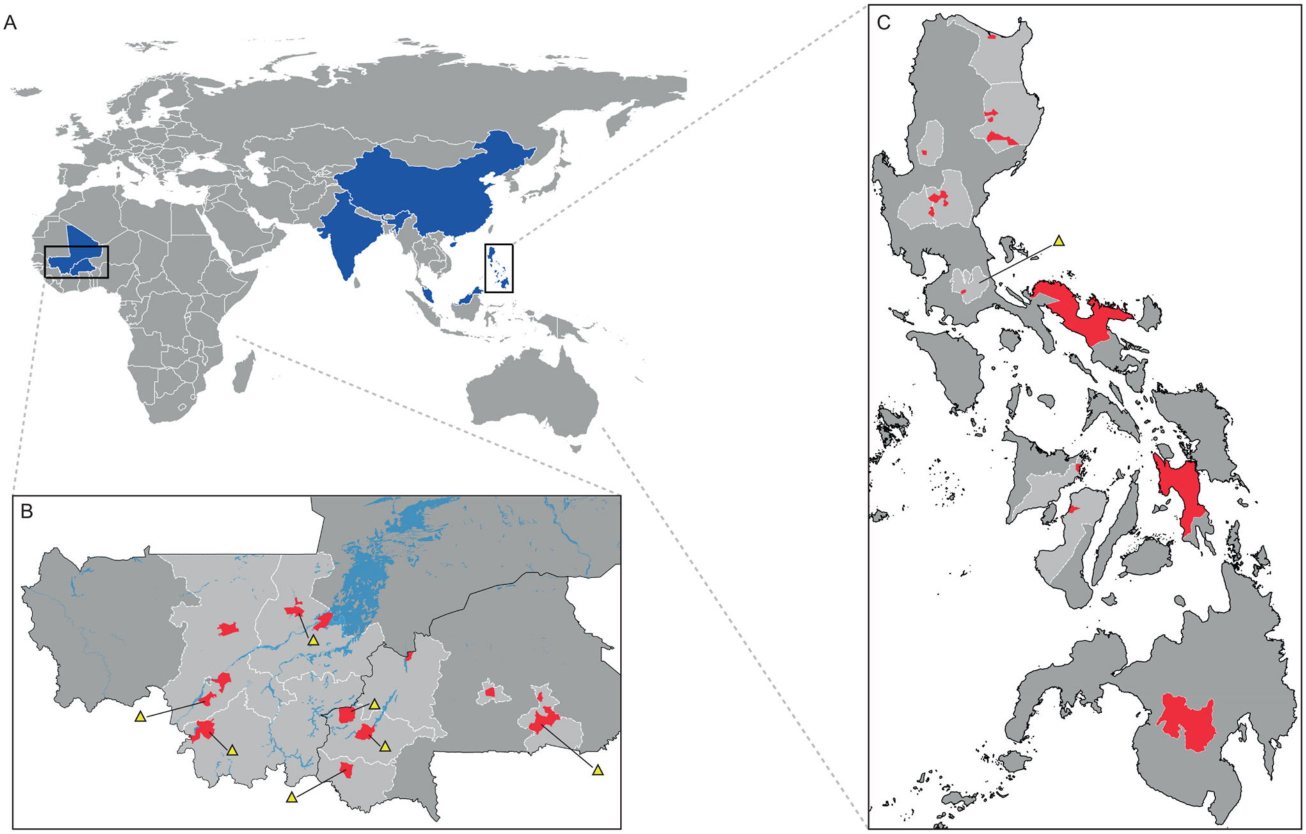

Fig. 3.

Origin of *Xanthomonas oryzae* pv. *oryzicola* isolates screened for the presence of the gibberellin (GA) biosynthetic operon. (a) Countries where *Xanthomonas oryzae* pv. *oryzicola* were isolated. Blue areas indicate Mali and Burkina Faso (West Africa) as well as India, Malaysia, China and the Philippines (Asia). Details on the collection area of West African (b) and Philippines (c) isolates. Red areas indicate provinces or towns (when available); yellow triangles indicate regions where isolates were found which do not have the GA biosynthetic operon.

Table 1

Total number of *Xanthomonas oryzae* pv. *oryzicola* isolates screened for the presence of the gibberellin (GA) biosynthetic operon, by country

Country	**Number of isolates**		
	total	**GA +**	**GA −**
Mali	35	31	4
Burkina Faso	32	27	5
Philippines	41	40	1
Malaysia	3	3	0
China	4	4	0
India	1	1	0
Total	**116**	**106**	**10**