RESEARCH LETTER

Genome-wide sequencing data reveals virulence factors implicated in banana *Xanthomonas* wilt

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Abstract

Banana *Xanthomonas* wilt is a newly emerging disease that is currently threatening the livelihoods of millions of farmers in East Africa. The causative agent is *Xanthomonas campestris* pathovar *musacearum* (*Xcm*), but previous work suggests that this pathogen is much more closely related to species *Xanthomonas vasicola* than to *X. campestris*. We have generated draft genome sequences for a banana-pathogenic strain of *Xcm* isolated in Uganda and for a very closely related strain of *X. vasicola* pathovar *vasculorum*, originally isolated from sugarcane, that is nonpathogenic on banana. The draft sequences revealed overlapping but distinct repertoires of candidate virulence effectors in the two strains. Both strains encode homologues of the *Pseudomonas syringae* effectors HopW, HopAF1 and RipT from *Ralstonia solanacearum*. The banana-pathogenic and non-banana-pathogenic strains also differed with respect to lipopolysaccharide synthesis and type-IV pili, and in at least several thousand single-nucleotide polymorphisms in the core conserved genome. We found evidence of horizontal transfer between *X. vasicola* and very distantly related bacteria, including members of other divisions of the *Proteobacteria*. The availability of these draft genomes will be an invaluable tool for further studies aimed at understanding and combating this important disease.

Introduction

Banana and plantain (*Musa* species) are a major crop in many tropical areas, especially Latin America, the Caribbean, South-East Asia and sub-Saharan Africa. They represent the most important food crop in Uganda, Rwanda and Burundi and are significant as a cash crop and staple food throughout the Great Lakes region of East Africa. Uganda is the second largest producer of bananas/plantains (after India) according to statistics from the Food and Agriculture Organisation of the United Nations (http://faostat.fao.org cited by Biruma *et al.*, 2007 and Vurro *et al.*, 2010). Since 2001, the emergence of banana *Xanthomonas* wilt (BXW) disease has threatened the livelihoods of tens of millions of East-African farmers (Tushemereirwe *et al.*, 2004; Biruma *et al.*, 2007). The disease has been known in Ethiopia on enset (*Ensete ventricosum*), a close relative of banana, since the 1960s (Shimelash *et al.*, 2008). However, BXW has recently spread to the Burundi, the Democratic Republic of Congo, Kenya, Rwanda, Tanzania and Uganda (Tushemereirwe *et al.*, 2004; Ndungo *et al.*, 2006; Biruma *et al.*, 2007; Reeder *et al.*, 2007; Carter *et al.*, 2010). The disease is characterized by premature ripening of fruits, internal brown discoloration of fruits and vascular tissues, wilting of bracts and male buds and progressive yellowing leading to complete wilting. Once established in an area, BXW spreads rapidly and often leads to complete loss of yield (Biruma *et al.*, 2007).

The etiologic agent of BXW is a Gram-negative bacterium, previously classified as *Xanthomonas campestris* pathovar *musacearum* (*Xcm*) (Young *et al.*, 1978). A recent phylogenetic study (Aritua *et al.*, 2008) suggested that rather than belonging to species *X. campestris*, the bacterium is more closely related to the species *Xanthomonas vasicola*, which includes pathovars *X. vasicola* pathovar *holcicola* (*Xvh*) pathogenic to sorghum and *X. vasicola* pathovar

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vasculorum (*Xvv*) pathogenic to sugarcane (*Saccharum officinarum*) and maize (*Zea mays*) (Ohobela & Claflin, 1987; Vauterin *et al*., 1992, 1995). Accordingly, *Xcm* can be considered as a new pathovar of species *X. vasicola* (Aritua *et al*., 2008). Aritua *et al*. (2008) also showed that strains of *Xvh* and *Xvv* were nonpathogenic on banana but were pathogenic on maize, whereas *Xcm* strains were pathogenic on both banana and maize. These pathogenicity data suggest a host-jump by a strain of *Xvh* or *Xvv* onto a *Musa* species, because the *Xcm* strains retained pathogenicity to maize (Aritua *et al*., 2008).

Xanthomonas is a genus within the *Gammaproteobacteria* that includes >20 species and hundreds of pathovars of Gram-negative rod-shaped plant-pathogenic bacteria (Vauterin *et al*., 1995). This genus includes causative agents of several economically important diseases. Complete genome sequences have been determined for several members of the genus (da Silva *et al*., 2002; Lee *et al*., 2005; Qian *et al*., 2005; Thieme *et al*., 2005; Salzberg *et al*., 2008; Vorholter *et al*., 2008; Pieretti *et al*., 2009; Moreira *et al*., 2010). However, no complete genome sequence is available for *X. vasicola* and little is known about the ecology and molecular genetics of the banana pathogen *Xcm* or its close relatives *Xvv* and *Xvh*.

We wish to understand the evolution of BXW and the genetic basis for the differing host specificities between *Xcm*, a pathogen of banana, and its close relative *Xvv*, a pathogen of sugarcane. Genetic differences may also reflect adaptations for epiphytic fitness and for dispersal, perhaps via insect vectors (Tinzaara *et al*., 2006; Mwangi *et al*., 2007; Shimelash *et al*., 2008) rather than virulence factors *per se*. Recent developments in DNA-sequencing technology provide opportunities for rapid and cost-effective comparative genomics studies (MacLean *et al*., 2009). We used the 'Next Generation' Illumina Solexa GA technology (Bentley *et al*., 2008) to generate draft genome sequences for banana-pathogenic *Xcm* NCPPB 4381 (*Xcm* 4381) and for the closely related *Xvv* NCPPB 702 (*Xvv* 702), which is not pathogenic on banana. Sequence analysis revealed several genetic differences between these two strains that might be important for host specificity, virulence and epiphytic fitness, including differences in the repertoires of secreted and translocated effector proteins, type IV pili (TFP) and enzymes for lipopolysaccharide biosynthesis.

Materials and methods

Xcm 4381 was originally isolated from banana in Uganda 2005. *Xvv* 702 was originally isolated from sugarcane in Zimbabwe in 1959. We obtained both strains from the National Collection of Plant Pathogenic Bacteria (NCPPB) at the Food and Environmental Research Agency (FERA, York, UK). Genomic DNA was prepared from cultures grown in NYGB (Nitrogen Yeast Glycerol Broth) medium using a Puregene Genomic DNA Purification Kit (Gentra Systems Inc., Minneapolis) and sequenced using the Illumina GA sequencing system. Illumina sequencing of genomic DNA and sequence assembly were performed as described previously (Studholme *et al*., 2009). We used MAQ (Li *et al*., 2008), BLAST (Altschul *et al*., 1990) and MUMMER (Delcher *et al*., 2002) for sequence alignment of short reads, contigs and whole genomes, respectively, and CGVIEW (Stothard & Wishart, 2005) for visualizing the alignments. SPLITSTREE (Huson, 1998) was used to build and draw phylogenetic trees. We deposited the draft genome data in GenBank under accession numbers ACHT00000000 (*Xcm* 4381) and ACHS00000000 (*Xvv* 702).

Results and discussion

Generation of genomic sequence data

We generated 5 052 905 pairs of 36-nucleotide reads from *Xcm* 4381; that is a total of 363 809 160 nucleotides, representing approximately 72 times 5 megabases, the typical genome size for *Xanthomonas* species. From *Xvv* 702, we generated 2 913 785 pairs of 36-nucleotide reads; that is a total of 209 792 520 nucleotides, representing approximately 42 times the expected genome size of 5 megabases. We assembled the Illumina data using the VELVET short-read assembly software (Table 1).

Comparison with genome sequences of other *Xanthomonas* species

The genome sequences of *Xcm* 4381 and *Xvv* 702 shared significantly greater nucleotide identity with each other than with the genome of any other sequenced *Xanthomonas*

Table 1. Summary statistics of *de novo* genome assemblies of paired 36-nucleotide Illumina genomic sequence reads from *Xanthomonas campestris* pathovar *musacearum* NCPPB 4381 (*Xcm* 4381) and *Xanthomonas vasicola* pathovar *vasculorum* NCPPB 702 (*Xvv* 702)

	Xcm 4381	*Xvv* 702
Number of scaffolds	115	97
Total length	4 789 105	5 475 474
Length of the longest scaffold	395 581	712 118
Mean scaffold length	41 644	56 448
Median scaffold length	6793	5459
N_{50} scaffold length	137 766	282 789
N_{50} scaffold number	11	6

Assembly was performed using VELVET 0.7.48 (Zerbino & Birney, 2008). Prediction and automated annotation of protein-coding genes was performed using FGENESB (http://tinyurl.com/fgenesb). N_{50} was calculated with respect to the predicted genome size of 5 megabases. That is, the 11 largest *Xcm* scaffolds were each at least 137 766 kb long and comprised a total of >2.5 megabases.

genome (Table 2). This is consistent with the previously reported close phylogenetic relationship between these strains (Aritua *et al.*, 2008).

The genome of a bacterial taxon can be divided into two compartments: a 'core genome' containing genes conserved in all the strains, and a 'dispensable genome' containing genes that are absent from one or more strains. Together, these two components make up the 'pan-genome' (Medini *et al.*, 2005). About 96% of the *Xcm* 4381 genome and 92% of the *Xvv* 702 genome are conserved between the two strains. However, these two genomes each contained several hundred kilobases of sequence that was not conserved (Table 2), representing part of the dispensable genome of the species *X. vasicola*. About 60–80% of the *Xcm* 4381 and *Xvv* 702 genomes were conserved in other sequenced *Xanthomonas* species. Figure 1 shows the sequence data from *Xcm* 4381 and *Xvv* 702 aligned against the genome of *Xanthomonas oryzae* pathovar *oryzae* MAFF 311018, revealing global patterns of conservation and variation. Alignment of the Illumina sequence reads vs. the *Xoo* genome also revealed 3011 high-confidence single-nucleotide polymorphisms (SNPs) between *Xcm* 4381 and *Xvv* 702 (see Supporting Information, Appendix S1).

Evidence of genetic exchange with distantly related bacteria

Several predicted proteins from *Xcm* 4381 and *Xvv* 702 most closely resembled sequences from bacteria not closely related to *Xanthomonas* species. Most of these proteins do not have a known function or have similarity to proteins encoded by phage, transposons or other mobile genetic elements. Many of those for which a function could be inferred were associated with plasmid replication, maintenance or transfer. For example, a 7.6-kb contig from *Xvv* 702 (GenBank: ACHS01000311.1) encoded several proteins with sequence similarity to proteins encoded by the gammaproteobacterium *Klebsiella pneumoniae* (Fouts *et al.*, 2008). Several of these proteins (Fig. 2a) share at least 80% amino acid sequence identity with *K. pneumoniae* plasmid-associated proteins RepA, TrbJ and TrbL. This gene cluster may have been horizontally transferred between a *Klebsiella* strain and an ancestor of *Xvv*, likely as part of a conjugative plasmid. *Klebsiella* species are often found as endophytic symbionts in plants, including sugarcane (Nunez & Colmer, 1968; Ando *et al.*, 2005; Govindarajan *et al.*, 2007) and so it is plausible that *Xanthomonas* and *Klebsiella* strains may come into close contact *in planta*.

Table 2. Comparison of the draft genome assembly of *Xcm* 4381 vs. *Xvv* 702 and previously published genome sequences

Aligned	Unaligned	Reference sequence	Aligned	Sequence identity
4 781 468 (99.99%)	676 (0.01%)	*Xcm* 4381	4 781 468 (99.99%)	100
4 610 859 (96.42%)	171 285 (3.58%)	*Xvv* 702	5 018 800 (92.51%)	98.99
3 465 165 (72.46%)	1 316 979 (27.54%)	*X. oryzae* pv. *oryzae* MAFF 311018 (RefSeq:NC_007705)	3 621 662 (73.31%)	91.85
3 466 993 (72.50%)	1 315 151 (27.50%)	*X. oryzae* pv. *oryzae* KACC10331 (RefSeq:NC_006834)	3 627 807 (73.42%)	91.84
3 474 319 (72.65%)	1 307 825 (27.35%)	*X. oryzae* pv. *oryzae* PXO99A (RefSeq:NC_010717)	3 774 095 (72.02%)	91.83
3 551 174 (74.26%)	1 230 970 (25.74%)	*X. oryzae* pv. *oryzicola* strain BLS256 (GenBank:AAQN00000000)	3 615 341 (74.82%)	91.82
3 782 289 (79.09%)	999 855 (20.91%)	*X. axonopodis* pv. *citri* strain 306 (RefSeq:NC_003919)	3 834 007 (74.08%)	91.04
3 795 103 (79.36%)	987 041 (20.64%)	*X. campestris* pv. *vesicatoria* strain 85-10 (RefSeq:NC_007508)	3 822 676 (73.82%)	91.02
3 703 057 (77.44%)	1 079 087 (22.56%)	*X. fuscans* ssp. *aurantifolii* ICPB 10535 (GenBank:ACPY00000000)	3 737 779 (74.57%)	90.96
3 665 866 (76.66%)	1 116 278 (23.34%)	*X. fuscans* ssp. *aurantifolii* ICPB 11122 (GenBank:ACPX00000000)	3 689 454 (75.61%)	90.91
2 788 675 (58.31%)	1 993 469 (41.69%)	*X. campestris* pv. *campestris* strain ATCC 33913 (RefSeq:NC_003902)	2 815 459 (55.46%)	87.38
2 781 610 (58.17%)	2 000 534 (41.83%)	*X. campestris* pv. *campestris* strain 8004 (RefSeq:NC_007086)	2 807 083 (54.52%)	87.37
2 791 015 (58.36%)	1 991 129 (41.64%)	*X. campestris* pv. *campestris* strain B100 (RefSeq:NC_010688)	2 824 723 (55.62%)	87.35
609 352 (12.74%)	4 172 792 (87.26%)	*X. albilineans* (RefSeq:NC_013722)	622 476 (16.52%)	84.57

Sequence identities were calculated using MUMMER (Delcher *et al.*, 2002).

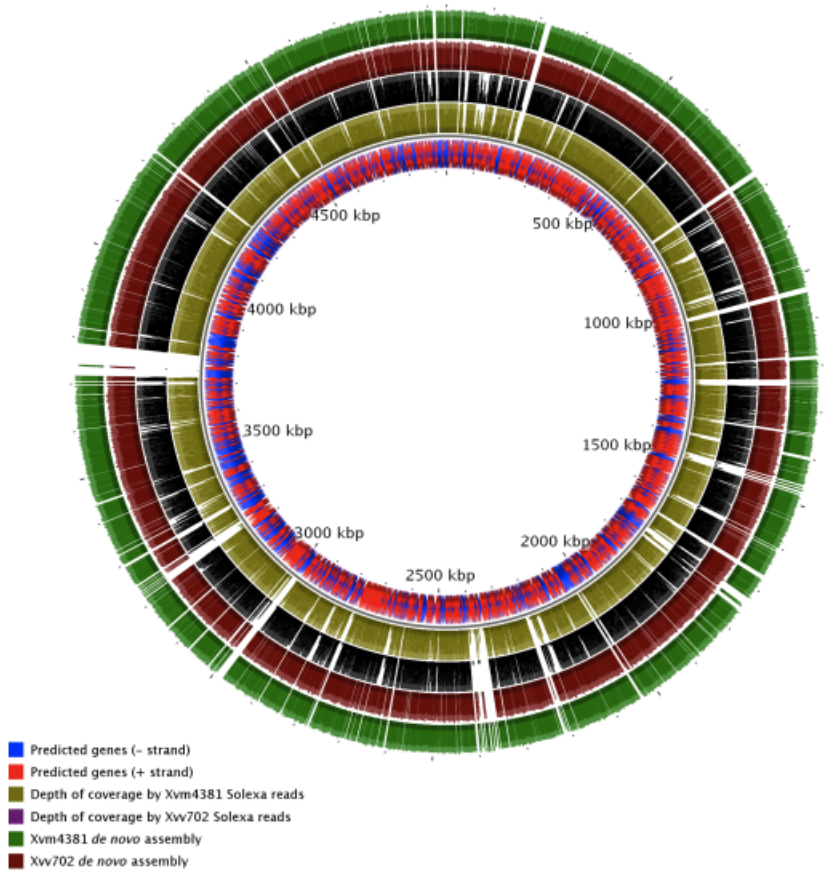

Fig. 1. Alignment of draft *Xcm* 4381 and *Xvv* 702 Illumina sequence data against *Xanthomonas oryzae* pathovar *oryzae* MAFF 311018. Illumina sequence reads were aligned against the reference genomes using MAQ (Li *et al.*, 2008). Scaffolds (from the VELVET *de novo* assemblies) were aligned against the reference genomes using BLASTN (Altschul *et al.*, 1990) with an *E*-value threshold of 1e − 10.

Xvv 702 harbours DNA sequence similar to that of plasmid pMRAD02 from the alphaproteobacterium *Methylobacterium radiotolerans* JCM2831 and to the broad-host-range plasmids pIPO2 and pSB102, which were isolated, respectively, from bacteria of the wheat rhizosphere (Tauch *et al.*, 2002; Mela *et al.*, 2008) and the alfalfa rhizosphere (Schneiker *et al.*, 2001). Opportunities for genetic exchange between *Xanthomonas* and *Methylobacterium* species might be common because the latter are often found to be associated with plants such as epiphytes, endophytes or symbionts (Pirttila *et al.*, 2000; Sy *et al.*, 2001; Idris *et al.*, 2006; Delmotte *et al.*, 2009; Podolich *et al.*, 2009).

We also found evidence of genetic exchange between *Xanthomonas* and *Betaproteobacteria*. A contig from *Xcm* 4381 (Fig. 2c) most closely resembled the genome of *Acidovorax* species JS42 (95% sequence identity over 7935 nucleotides) and, slightly more distantly (94% identity over 3327 nucleotides), resembled the genome of *X. campestris* pathovar *vesicatoria* 85-10. This region encodes a predicted TrbK-like protein. TrbK is usually plasmid associated (Haase *et al.*, 1996), but the corresponding genomic regions in *Acidovorax* species JS42 and in *X. campestris* pathovar *vesicatoria* 85-10 appear to be chromosomally located. It is unclear whether the 23-kb *Xcm* 4381 contig (Fig. 2c) represents a plasmid or is part of the chromosome.

Type III secretion system (T3SS) effectors

Plant-pathogenic *Xanthomonas* pathovars require a T3SS to secrete and translocate effector proteins (Alfano & Collmer, 2004; Yang *et al.*, 2005; Grant *et al.*, 2006; Gurlebeck *et al.*, 2006; White *et al.*, 2006, 2009; Kay & Bonas, 2009; Buttner & Bonas, 2010) in order to cause disease. These effectors have evolved to manipulate host cellular processes to the benefit of the pathogen; however, many plants have evolved resistance whereby they can recognize specific effectors, triggering the hypersensitive response. Therefore, in the context of a resistant plant, these effectors show an 'avirulence' activity, thus limiting the pathogen's host range (Alfano & Collmer, 2004; Yang *et al.*, 2005; Grant *et al.*, 2006; Gurlebeck *et al.*, 2006; White *et al.*, 2006, 2009; Kay & Bonas, 2009; Buttner & Bonas, 2010). A single *Xanthomonas* genome typically encodes 20–30 T3SS effectors. The repertoire of effectors varies between species and strains within species and is believed to be a key determinant in the host range of a given pathogen.

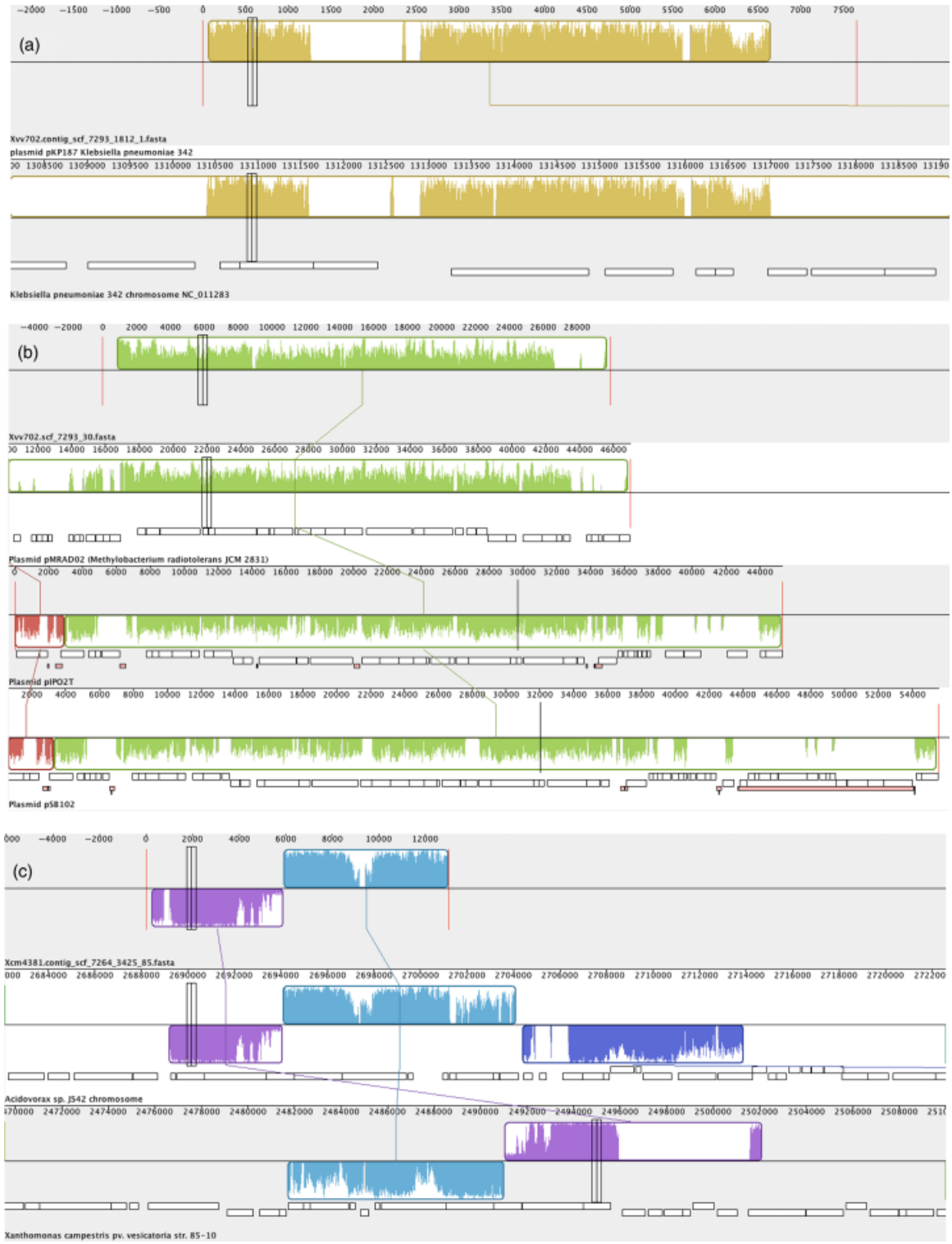

Fig. 2. Evidence of recent horizontal transfer with distantly related bacteria. (a) A 7.6-kb contig (GenBank: ACHS01000311.1) from the *Xvv* 702 genome assembly aligned against the chromosome of *Klebsiella pneumoniae* strain 342 (Fouts *et al*., 2008). (b) A 30-kb scaffold (GenBank: GG699237.1) from the *Xvv* 702 assembly aligned against plasmid pMRAD02 from the alphaproteobacterium *Methylobacterium radiotolerans* JCM2831 and broad-host-range plasmids pIPO2 and pSB102. (c) A contig (GenBank: ACHT01000374.1) from the *Xcm* 4381 assembly aligned against the chromosome of *Acidovorax* species strain JS42 and the chromosome of *Xanthomonas campestris* pathovar *vesicatoria* 85-10.

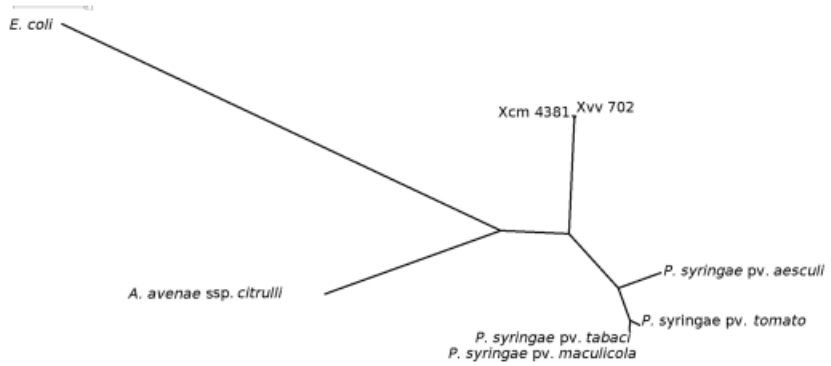

Fig. 3. *Xcm* 4381 and *Xvv* 702 encode homologues of the *Pseudomonas syringae* effector HopW1. The neighbour-joining phylogenetic tree was built using SPLITSTREE (Huson, 1998) from an alignment of the following sequences: GI:289668075 (*Xcm* 4381), GI:289664497 (*Xvv* 702), GI:213971766 (*P. syringae* pathovar *tomato* strain T1), GI:257485818 (*P. syringae* pathovar *tabaci* strain ATCC11528), GenBank: AAT35179.1 (*P. syringae* pathovar *maculicola*), GenBank: ABM32137.1 (*Acidovorax avenae* ssp. *citrulli* AAC00-1), GI:289650598 (*P. syringae* pathovar *aesculi* strain 2250) and GenBank: BAI30858.1 (*Escherichia coli* O103:H2 strain 12009).

The draft genomes of both *Xcm* 4381 and *Xvv* 702 encoded a complete T3SS apparatus. To identify homologues of known T3SS effectors, we used BLAST searches against catalogues of proteins from the *Pseudomonas syringae* Hop Identification and Nomenclature Home Page (http://www.pseudomonas-syringae.org/), The *Xanthomonas* Resource (http://www.xanthomonas.org/t3e.html) and papers by White *et al.* (2009) and Gurlebeck *et al.* (2006). In common with all previously sequenced *Xanthomonas* genomes, both draft genomes encode homologues of the candidate T3SS effectors AvrBs2, AvrGf1, XopF, XopK, XopL, XopN, XopP, XopQ, XopR, XopX and XopZ. Both strains also encode homologues of XopA, XopB, XopG, XopH, XopI, XopY, XopAA, XopAD, XopAE and XopAK, which are conserved in a subset of the previously sequenced *Xanthomonas* genomes (http://www.xanthomonas.org/t3e.html). Both *Xcm* 4381 and *Xvv* 702 also encode proteins sharing 71% amino acid sequence identity with *P. syringae* effector HopW1; these have no significant sequence similarity to any known *Xanthomonas* protein (Fig. 3). Both draft genomes contained genes encoding homologues of the *P. syringae* effector HopAF1 (GI:289668103 and GI:289664422); these are located in the integron locus adjacent to *ilvD* (Gillings *et al.*, 2005). Both genomes also encode proteins (GI:289669426 and GI:289663837) sharing 30% amino acid sequence identity with the putative T3SS effector RipT (RSc3212), a YopT-like cysteine protease from the betaproteobacterium *Ralstonia solanacearum* GMI1000 (Poueymiro & Genin, 2009). Close homologues are not found in any other *Xanthomonas* genomes, but a protein (GI:270492983) from another plant-pathogenic betaproteobacterium, *Acidovorax avenae* ssp. *avenae* ATCC 19860, shares 48% sequence identity with the *Xvv* and *Xcm* RipT-like proteins.

There are some differences between *Xcm* 4381 and *Xvv* 702 with respect to their complements of effectors that might contribute to their different host ranges. *Xcm* 4381 encodes two predicted YopJ-like C55 cysteine proteases (GI:289670655 and GI:289671144) that are absent from *Xvv* 702. On the other hand, *Xvv* 702 encodes a protein (GI:289661936) sharing 87% amino acid sequence identity with *Xanthomonas euvesicatoria* XopAF (also known as AvrXv3) (Astua-Monge *et al.*, 2000). This gene is absent from *Xcm* 4381, but shares 35% identity (at the amino acid level) with the HopAF1-like genes found at the integron locus in both *Xcm* and *Xvv*. Such differences in effector repertoires have previously been shown to be significant for host adaptation (Wei *et al.*, 2007; Kvitko *et al.*, 2009; Lindeberg *et al.*, 2009). For example, HopQ1-1 is present in *P. syringae* pathovar *phaseolicola*, where it suppresses immunity in beans, but is absent from *P. syringae* pathovar *tabaci*, and triggers defences in tobacco (Ferrante *et al.*, 2009). It is possible that the differences in effector repertoires of *Xcm* 4381 and *Xvv* 702 are significant for the adaptation of *Xcm* 4381 to a new host (i.e. banana). It remains to be tested whether the two *Xcm* 4381 YopJ- and HopR-like proteins suppress defences and whether the *Xvv* 702 AvrXv3 confers avirulence in banana.

Xcm 4381 and *Xvv* 702 differ in their lipopolysaccharide biosynthetic pathways

The outer membranes of Gram-negative bacteria are covered with lipopolysaccharides (Lerouge & Vanderleyden, 2002). Among different strains of *X. campestris* pathovar *campestris* and *X. oryzae* pathovar *oryzae*, the lipopolysaccharide biosynthesis locus shows hypervariability arising from horizontal transfer (Patil & Sonti, 2004; Patil *et al.*,

2007). The lipopolysaccharide locus in *Xcm* 4381 (GenBank: ACHT01000245.1) most closely matches that of *Xanthomonas axonopodis* pathovar *citri* 306 (93% nucleotide sequence identity). The lipopolysaccharide locus in *Xvv* 702 (GenBank: ACHS01000380.1) shows no significant sequence similarity to that of *Xcm* 4381. It does, however, share 86% nucleotide sequence identity with *Xanthomonas albilineans* strain GPE PC73 (Pieretti *et al.*, 2009). This is incongruent with the close phylogenetic relationship between *Xcm* 4381 and *Xvv* 702 and indicates recent horizontal transfer in one or both strains from independent sources. Any significance of this variation between *Xcm* 4381 and *Xvv* 702 for virulence and host specificity remains unclear. The lipopolysaccharides can be an important virulence factor in plant pathogens (Hendrick & Sequeira, 1984; Drigues *et al.*, 1985; Jayaswal *et al.*, 1985; Schoonejans *et al.*, 1987; Kao & Sequeira, 1991; Kingsley *et al.*, 1993; Dow *et al.*, 1995; Titarenko *et al.*, 1997). On the other hand, they can also act as a pathogen-associated molecular pattern, recognized by the plant and triggering specific defenses (oxidative burst, increased levels of intracellular calcium, modifications to cell wall) (Dow *et al.*, 2000; Meyer *et al.*, 2001). Therefore, it has been argued that variation in lipopolysaccharides might be expected as a means of avoiding recognition in plant defense (Patil *et al.*, 2007). However, *X. campestris* pathovar *vesicatoria*, and presumably other xanthomonads, can suppress lipopolysaccharide-triggered responses through secretion of effectors via the T3SS (Keshavarzi *et al.*, 2004), suggesting that avoidance of recognition by the plant might be less important. Alternatively, the driver for variation might be interactions with phage (Keshavarzi *et al.*, 2004) or with insect vectors (Pal & Wu, 2009).

Xcm 4381 and *Xvv* 702 encode different TFP

Functions of TFP include twitching motility (Liu *et al.*, 2001; Mattick, 2002; De La Fuente *et al.*, 2007; Li *et al.*, 2007, 2010; Pelicic, 2008; Bahar *et al.*, 2009) and attachment (Jenkins *et al.*, 2005; Heijstra *et al.*, 2009), meaning that they often play a role in virulence as well as contributing to survival and epiphytic fitness before infection (Roine *et al.*, 1998; Shime-Hattori *et al.*, 2006; Darsonval *et al.*, 2008; Varga *et al.*, 2008). An 8-kb gene cluster in *Xcm* 4381 (GenBank: ACHT01000072.1) encodes TFP components FimT, PilV, PilW, PilX, PilY1 and PilE. This cluster is adjacent to a tRNA-Asn gene. Nucleotide sequence alignments using MAUVE (Darling *et al.*, 2004) revealed that in previously sequenced genomes this TFP-encoding gene cluster was either completely absent or partially deleted and interrupted by transposon-associated sequences. For example, in *Xcv* 85-10, *pilX* appears to be replaced by an IS1477 transposase (GenBank: CAJ24495.1). In *Xoo* KACC10331, it is replaced by a different putative transposase (GenBank: YP_201837.1). The observation that this TFP gene cluster is uniquely intact in *Xcm* 4381 suggests that in this strain, unlike other sequenced *Xanthomonas* strains where it is apparently dispensable, the encoded TFP may have some adaptive function.

A different gene cluster in *Xvv* 702 (GenBank: ACHS01000345.1) encodes homologues of TFP components FimT, PilE, PilY1, PilW and PilV. This region is conserved in the sequenced genomes of *X. oryzae* pathovar *oryzae* but not in *Xcm* 4381. The respective TFP clusters may be functionally redundant. However, there is little sequence similarity between proteins, respectively encoded on the *Xvv* 702 and the *Xcm* 4381 TFP clusters. These sequence differences likely translate into differences in physicochemical properties of the resulting TFP systems, including differences in glycosylation (Darling *et al.*, 2004). These differences might be adaptive for attachment to and motility on different plant surfaces.

Concluding remarks

We have used the Illumina GA platform to rapidly generate genome-wide sequence data for *Xcm*, the causative agent of BXW, and a closely related strain, *Xvv* 702, which is unable to infect banana. The draft genome assemblies reveal a core repertoire of T3SS effectors and other candidate virulence factors and will serve as a starting point for molecular studies of this recently emerging disease. Comparison between *Xcm* 4381 and *Xvv* 702 revealed a catalogue of genetic differences, a subset of which presumably underlies the host-jump onto banana. These differences include dispensable genes that are present in one strain and absent from the other as well as several thousand SNPs in the core genome. Among the dispensable genes were those encoding several T3SS effectors and TFP. We also discovered evidence of recent horizontal genetic transfer between *Xvv* and distantly related bacteria, including members of other divisions of the *Proteobacteria*, that may be significant for the evolution of new disease. We hope that the availability of these draft genome sequences will stimulate further work towards understanding and eventually eradicating this devastating disease.

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Authors' contribution

J.S. and J.D.G.J. contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of single-nucleotide polymorphisms in Xvv 702 and Xcm 4381 with respect to the published genome sequence of Xoo MAFF 311018.

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