The complete genome sequence of *Xanthomonas albilineans* provides new insights into the reductive genome evolution of the xylem-limited *Xanthomonadaceae*

Isabelle Pieretti†1, Monique Royer†1, Valérie Barbe2, Sébastien Carrère3, Ralf Koebnik4, Stéphane Cociancich1, Arnaud Couloux2, Armelle Darrasse5, Jérôme Gouzy3, Marie-Agnès Jacques5, Emmanuelle Lauber3, Charles Manceau5, Sophie Mangenot2, Stéphane Poussier6, Béatrice Segurens2, Boris Szurek4, Valérie Verdier4, Matthieu Arlat7 and Philippe Rott*1

Address: 1CIRAD, UMR 385 BGPI, Campus international de Baillarguet, TA A-54K, F-34398 Montpellier Cedex 5, France, 2Génoscope, Centre national de séquençage, CEA/DSV/IG/Genoscope, 2 rue Gaston Cremieux, F-91057 Evry Cedex, France, 3Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR CNRS-INRA 2594/441, F-31320 Castanet-Tolosan, France, 4Laboratoire Génome et Développement des Plantes, Université de Perpignan via Domitia - CNRS - IRD, UMR 5096, 911 Avenue Agropolis, BP 64501, F-34394 Montpellier cedex 5, France, 5INRA, UMR 077 PaVé, F-49071 Beaucouzé, France, 4Agrocampus Ouest centre d’Angers, UMR 077 PaVé, F-49071 Beaucouzé, France and 7Université de Toulouse, UPS, 118 Route de Narbonne, F-31062 Toulouse, France

Email: Isabelle Pieretti - isabelle.pieretti@cirad.fr; Monique Royer - monique.royer@cirad.fr; Valérie Barbe - vbarbe@genoscope.cns.fr; Sébastien Carrère - Sebastien.Carrere@toulouse.inra.fr; Ralf Koebnik - Ralf.Koebnik@mpl.ird.fr; Stéphane Cociancich - stephane.cociancich@cirad.fr; Arnaud Couloux - acouloux@genoscope.cns.fr; Armelle Darrasse - Armelle.Darrasse@angers.inra.fr; Jérôme Gouzy - gouzy@toulouse.inra.fr; Marie-Agnès Jacques - Marie-Agnes.Jacques@angers.inra.fr; Emmanuelle Lauber - Emmanuel.Lauber@toulouse.inra.fr; Charles Manceau - charles.manceau@angers.inra.fr; Sophie Mangenot - mangenot@genoscope.cns.fr; Stéphane Poussier - Stephane.Poussier@agrocampus-ouest.fr; Béatrice Segurens - segurens@genoscope.cns.fr; Boris Szurek - boris.szurek@mpl.ird.fr; Valérie Verdier - Valerie.Verdier@ird.fr; Matthieu Arlat - Matthieu.Arlat@toulouse.inra.fr; Philippe Rott* - philippe.rott@cirad.fr

* Corresponding author    †Equal contributors

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**Abstract**

**Background:** The *Xanthomonadaceae* family contains two xylem-limited plant pathogenic bacterial species, *Xanthomonas albilineans* and *Xylella fastidiosa*. *X. fastidiosa* was the first completely sequenced plant pathogen. It is insect-vectored, has a reduced genome and does not possess *hrp* genes which encode a Type III secretion system found in most plant pathogenic bacteria. *X. fastidiosa* was excluded from the *Xanthomonas* group based on phylogenetic analyses with rRNA sequences.

**Results:** The complete genome of *X. albilineans* was sequenced and annotated. *X. albilineans*, which is not known to be insect-vectored, also has a reduced genome and does not possess *hrp* genes. Phylogenetic analysis using *X. albilineans* genomic sequences showed that *X. fastidiosa* belongs to the *Xanthomonas* group. Order of divergence of the *Xanthomonadaceae* revealed that *X. albilineans* and *X. fastidiosa* experienced a convergent reductive genome evolution during their descent from the progenitor of the *Xanthomonas* genus. Reductive genome evolutions of the two xylem-limited species are discussed in detail.
Xanthomonadaceae were compared in light of their genome characteristics and those of obligate animal symbionts and pathogens.

**Conclusion:** The two xylem-limited Xanthomonadaceae, during their descent from a common ancestral parent, experienced a convergent reductive genome evolution. Adaptation to the nutrient-poor xylem elements and to the cloistered environmental niche of xylem vessels probably favoured this convergent evolutionary change. However, genome characteristics of *X. albilineans* differ from those of *X. fastidiosa* and obligate animal symbionts and pathogens, indicating that a distinctive process was responsible for the reductive genome evolution in this pathogen. The possible role in genome reduction of the unique toxin albicidin, produced by *X. albilineans*, is discussed.

**Background**

The Xanthomonadaceae are a family of Gram negative bacteria belonging to the order Xanthomonadales in the gamma subdivision of the Proteobacteria [1]. Members of this family are typically characterized as environmental organisms and occupy diverse ecological niches, such as soil and water, as well as plant tissues. Many Xanthomonadaceae, especially species from the genera Xanthomonas and Xylella, cause plant diseases and only one, *Stenotrophomonas maltophilia*, is known to be an opportunistic human pathogen.

Complete genome sequences of several Xanthomonas species and *Xylella fastidiosa* strains have been determined, making those bacteria attractive models for study of plant-pathogen interactions [2]. *X. fastidiosa* was the first completely sequenced plant pathogen. Sequence analysis showed that this xylem-limited bacterium, which is insect-vectored to a variety of diverse hosts, had a reduced genome and did not possess *hrp* genes, which encode a Type III secretion system (T3SS) found in most Gram negative plant pathogenic bacteria [3]. Phylogenetic analysis with rRNA sequences showed that the two major genera of Xanthomonadaceae, Xanthomonas and *Stenotrophomonas*, form a coherent group excluding *X. fastidiosa* [4-6]. These characteristics suggested the hypothesis that this species evolved from an ancestor shared with Xanthomonas and *Stenotrophomonas* by genome reduction during adaptation to life within its hosts [2].

*Xanthomonas albilineans* is a systemic, xylem-limited pathogen that causes leaf scald, one of the major diseases of sugarcane (interspecific hybrids of *Saccharum* spp.) [7]. Leaf scald symptoms vary from a single, white, narrow, sharply defined stripe to complete wilting and necrosis of infected leaves, leading to plant death. *X. albilineans* produces the toxin albicidin that has phytotoxic and antibiotic properties [8]. Albicidin is a potent DNA gyrase inhibitor that targets the chloroplastic DNA gyrase A, inhibits chloroplast DNA replication and blocks chloroplast differentiation, resulting in the white foliar stripe symptoms [8,9]. All attempts to identify *hrp* genes in *X. albilineans* failed so far [10,11]. A phylogenetic study with the housekeeping genes *ihfA* and *efp*, which did not include *S. maltophilia* sequences, suggested that *X. albilineans* was an evolutionary intermediate between several Xanthomonas species and *X. fastidiosa* [11].

Unlike other xylem-invading xanthomonads that interact with living plant tissues, such as *X. campesris* pv. *campesris* or *X. oryzae* pv. *oryzae*, *X. fastidiosa* and *X. albilineans* appear to be strictly xylem-limited, living only in dead xylem cells or tracheary elements. In order to better understand the evolution of these two xylem-limited Xanthomonadaceae, we sequenced the genome of *X. albilineans* strain GPE PC73 from Guadeloupe [11]. This sequence was compared to complete genome sequences of other closely related members of the Xanthomonadaceae. This comparative analysis revealed that *X. albilineans* and *X. fastidiosa* experienced a convergent reductive genome evolution from a common ancestral parent of the Xanthomonas genus.

**Results**

**General genomic features of *X. albilineans***

The genome of *X. albilineans* strain GPE PC73 consists of one circular chromosome of 3,768,695 bp and three extrachromosomal plasmids of 32, 27 and 25 Kbp, respectively. The chromosome exhibits a GC skew pattern typical of prokaryotic genomes that have two major shifts, one near the origin and one near the terminus of replication, with *dnaA* assigned as base pair 1 of the chromosome (Figure 1). The GC skew pattern of *X. albilineans* contains a lower number of diagram distortions and has much lower amplitude than the GC skew pattern of *X. fastidiosa* (Figure 2). However, the amplitude of the GC skew pattern of *X. albilineans* is significantly higher than the one of other xanthomonads and *S. maltophilia* (Figure 2). Of the 3115 putative protein-coding sequences (CDSs) manually annotated on the chromosome of *X. albilineans* strain GPE PC73, 2014 (64%) were assigned putative functions based on homology to other known proteins and domain analyses.

The general features of the *X. albilineans* chromosome were compared to those of the chromosomes of the fol-
Evidence of convergent genome reductive evolution of X. albilineans and X. fastidiosa

The phylogenetic tree presented in Figure 3 suggests that X. albilineans, X. fastidiosa and the Hrp Xanthomonas group
derived from a common ancestor shared with *Stenotrophomonas*. The chromosome sizes of *X. albilineans* and *X. fastidiosa* are smaller than those of any other xanthomonad (Table 1), suggesting that both species evolved from the progenitor of xanthomonads by genome reduction. To examine how the chromosomes of *X. albilineans* and *X. fastidiosa* have evolved to result in these different sizes, we determined the order of divergence of *X. albilineans*, *X. fastidiosa*, the four xanthomonads of the Hrp *Xanthomonas* group and *S. maltophilia* (Figure 3). Orthologous genes shared by *S. maltophilia* and a species of the Hrp *Xanthomonas* group, but missing in *X. albilineans* or *X. fastidiosa*, may be assumed to have been inherited from the progenitor of the *Xanthomonas* genus. On the basis of OrthoMCL analysis, the numbers of genes lost by *X. albilineans* strain GPE PC73 and *X. fastidiosa* strain 9a5c are at least 585 and 1121, respectively (numbers obtained by comparison with *X. campestris* pv. *campestris*; 585 genes lost by *X. albilineans* = the number of CDSs conserved in both *X. campestris* pv. *campestris* and *S. maltophilia* and missing in *X. albilineans*; 1121 genes lost by *X. fastidiosa* = the number of CDSs conserved in both *X. campestris* pv. *campestris* and *S. maltophilia* and missing in *X. fastidiosa*; Figure 4A). The number of ancestral genes inherited from the progenitor of the *Xanthomonas* genus is higher in *X. axonopodis* pv. *vesicatoria* than in any other species of the Hrp *Xanthomonas* group (2809 ancestral genes present in *X. axonopodis* pv. *vesicatoria* = the number of CDSs of *X. axonopodis* pv. *vesicatoria* strain 85-10; *X. campestris* pv. *campestris* strain ATCC 33913 and *X. axonopodis* pv. *citri* strain 306 (data not shown). A GC skew pattern very similar to that of *S. maltophilia* strain R551-3 was observed for *S. maltophilia* strain K279a (data not shown).
Table 1: General features of nine Xanthomonadaceae chromosomes

| Features                        | X. oryzae pv. oryzae strain MAFF 311018 | X. campesiris pv. campesiris strain ATCC 33913 | X. axonopodis pv. vescatoria strain 85-10 | X. axonopodis pv. citri strain 306 | S. maltophilia strain K279a | S. maltophilia strain 85-10 | X. fastidiosa strain 9a5c | X. fastidiosa strain Temecula1 | X. albilineans strain GPE PC73 |
|--------------------------------|----------------------------------------|-----------------------------------------------|------------------------------------------|----------------------------------|----------------------------|---------------------------|---------------------------|-----------------------------|-----------------------------|
| Size (bp)                      | 4,940,217                               | 5,076,187                                     | 5,178,466                                | 5,175,554                        | 4,851,126                  | 4,573,969                  | 2,679,306                   | 2,519,802                    | 3,768,695                   |
| G+C content (%)                | 63                                      | 65                                             | 65                                       | 64                                | 66                        | 66                        | 52                         | 51                          | 63                          |
| Coding density (%)             | 83                                      | 84                                             | 87                                       | 84                                | 88                        | 89                        | 83                         | 79                          | 84                          |
| Protein-coding sequences (CDS) | 4372                                    | 4181                                           | 4487                                     | 4312                             | 4386                      | 4039                      | 2766                      | 2123                        | 3115                        |
| Average length of all CDS (bp) | 948                                     | 1027                                           | 1005                                     | 1032                             | 980                       | 1010                      | 805                        | 964                         | 1059                        |
| Average length of the core genome CDS (bp) | 1055                                   | 1058                                           | 1060                                     | 1056                             | 1051                      | 1048                      | 1048                      | 1044                        | 1050                        |
| CDS < 300 bp                   | 346                                     | 318                                            | 428                                      | 299                              | 294                       | 261                       | 738                        | 194                         | 283                         |
| rRNA operons                   | 2                                       | 2                                              | 2                                        | 2                                | 4                         | 4                         | 2                          | 2                           | 2                           |
| tRNA                           | 54                                      | 54                                             | 54                                       | 54                                | 74                        | 71                        | 49                         | 49                          | 51                          |

In these analyses, we selected the genome of X. axonopodis pv. vescatoria, which contains the highest number of genes inherited from the progenitor of the Xanthomonas genus (Figure 4A). OrthoMCL analysis identified 3004 CDSs of X. axonopodis pv. vescatoria that do not include any transposase genes and that are shared either by X. albilineans strain GPE PC73, X. fastidiosa strain 9a5c or one of the two S. maltophilia strains K279a and R551-3. For each of the 3004 CDSs, we looked for the best BLAST hit within a database that included: (i) all annotated CDSs from the genome sequence of X. albilineans strain GPE PC73 and X. fastidiosa strain 9a5c, 512 ancestral genes were lost by both X. albilineans and X. fastidiosa (512 = number of orthologs shared only by X. axonopodis pv. vescatoria strain 85-10 and S. maltophilia strain R551-3, Figure 4B), 960 ancestral genes were lost only by X. fastidiosa (960 = 613 + 290 + 57 = number of CDSs of X. albilineans strain GPE PC73 conserved in X. axonopodis pv. vescatoria strain 85-10 or S. maltophilia strain R551-3 and missing in X. fastidiosa strain 9a5c, Figure 4B), and 182 ancestral genes were lost only by X. albilineans (182 = 63 + 39 + 80 = number of CDSs of X. fastidiosa strain 9a5c conserved in X. axonopodis pv. vescatoria strain 85-10 or S. maltophilia strain R551-3 and missing in X. albilineans strain GPE PC73, Figure 4B).

Comparison of the reductive evolutions of X. albilineans and X. fastidiosa

Further comparative analyses were performed to compare genome erosion in X. albilineans and X. fastidiosa. For these analyses, we selected the genome of X. axonopodis pv. vescatoria, which contains the highest number of genes inherited from the progenitor of the Xanthomonas genus (Figure 4A). OrthoMCL analysis identified 3004 CDSs of X. axonopodis pv. vescatoria that do not include any transposase genes and that are shared either by X. albilineans, X. fastidiosa or S. maltophilia. We made the hypothesis that these genes were inherited by X. axonopodis pv. vescatoria from the ancestor of the xanthomonads. These best BLAST hit analyses confirmed that 2864 of these 3004 CDSs have the same ancestor as genes present either in X. albilineans, X. fastidiosa or S. maltophilia and were therefore inherited by X. axonopodis pv. vescatoria from the progenitor of the Xanthomonas genus. The elimination of paralogs present in at least two copies in X. axonopodis pv. vescatoria generated a list of 2816 CDSs representing one copy of each gene inherited by X. axonopodis pv. vescatoria from the progenitor of the Xanthomonas genus. These
2816 CDSs are listed and individually analysed in additional file 1. Among them, 1334 CDSs are shared by both *X. fastidiosa* and *X. albilineans* (these represent the ancestral genes conserved by *X. fastidiosa* and *X. albilineans*), 480 CDSs are shared only with one of the two *S. maltophilia* strains (they represent the ancestral genes lost by both *X. fastidiosa* and *X. albilineans*), 112 CDSs are shared with *X. fastidiosa* but not with *X. albilineans* (they represent the genes lost only by *X. fastidiosa*). The distribution in functional COG categories of these 2816 CDSs is illustrated in Figure 5.

Analyses of the arrangement of these 2816 ancestral genes on the chromosome of *X. axonopodis* pv. *vesicatoria* strain 85-10 led to the identification of DNA regions constituting contiguous ancestral genes that are missing in *X. albilineans* or *X. fastidiosa* (Additional file 1). During the speciation of *X. fastidiosa* or *X. albilineans*, the loss of these DNA regions was due either to a single event of deletion or to the cumulative effect of multiple events (pseudog-
Figure 4

Venn diagrams showing the number of orthologous CDSs as determined by OrthoMCL analyses among strains of Xanthomonadaceae. (A) Venn diagrams showing the number of orthologous CDSs among (i) X. albilineans strain GPE PC73 (XAL), S. maltophilia strain R551-3 (SMA) and one of the four following Hrp Xanthomonas strains: X. campestris pv. campes- 

stris strain ATCC 33913 (XCC), X. axonopodis pv. vesicatoria strain 85-10 (XCV), X. axonopodis pv. citri strain 306 (XAC) or X. oryzae pv. oryzae strain MAFF 311018 (XOO), and (ii) X. fastidiosa strain 9a5c (XYL), S. maltophilia strain R551-3 (SMA) and one of the four following Hrp Xanthomonas strains: X. campestris pv. campesstris strain ATCC 33913 (XCC), X. axonopodis pv. vesicatoria strain 85-10 (XCV), X. axonopodis pv. citri strain 306 (XAC) or X. oryzae pv. oryzae strain MAFF 311018 (XOO). The number of predicted ancestral CDSs of respectively XCC, XCV, XAC and XOO (CDSs conserved in SMA, XAL or XYL) are underlined and the total number of these predicted ancestral CDSs is indicated below each Venn diagram. (B) Venn diagram showing the number of orthologous CDSs among (i) X. albilineans strain GPE PC73 (XAL), S. maltophilia strain R551-3 (SMA), X. axonopodis pv. vesicatoria strain 85-10 (XCV) and X. fastidiosa strain 9a5c (XYL). Numbers do not include paralogous CDSs.
enization and short deletions). For example, the loss in *X. fastidiosa* of the large DNA region constituted by ancestral genes from XCV1928 to XCV2044 seems to be due to a single event of deletion since all these ancestral genes are missing in *X. fastidiosa* (Additional file 1). This large DNA region lost by *X. fastidiosa* encodes all flagellar proteins and several chemotaxis proteins. The sum of the length of the ancestral genes present in this DNA region is 106,626 bp, strongly suggesting that *X. fastidiosa* experienced a single deletion of a DNA fragment of a larger size. The other DNA regions constituting contiguous ancestral genes that are missing in *X. albilineans* or *X. fastidiosa* are shorter and, for this reason, their loss may result either from a single event of deletion or from multiple mutational events. Analysis of the arrangement on the chromosome of *X. axonopodis* pv. *vesicatoria* strain 85-10 of ancestral genes absent in *X. albilineans* or *X. fastidiosa* did not allow us to determine if the genes absent in both xylem-limited Xanthomonadaceae were lost by their common ancestor or were lost independently after their divergence.

The loss of genes by pseudogenization and short deletions should not affect the position on the chromosome of the genes that precede or follow the lost genes. In order to identify ancestral genes putatively lost by pseudogenization and short deletions, we looked for ancestral lost genes that are present on the chromosome of *X. axonopodis* pv. *vesicatoria* between the orthologs of two ancestral genes that are contiguous and conserved in *X. fastidiosa* or *X. albilineans*. For example, the *rpf* (for regulation of pathogenicity factors) gene cluster contains in *X. axonopodis* pv. *vesicatoria* two ancestral genes (XCV1913 and XCV1914 which are conserved in *S. malophilia* which are missing in both *X. albilineans* and *X. fastidiosa*). The ancestral genes XCV1912 and XCV1915 that precede and follow respectively these two lost genes are orthologs of either Xalc_1342 and Xalc_1343 or XFl110 and XFl111 that are contiguous in *X. albilineans* and *X. fastidiosa*, respectively. Using the same strategy we identified 147 and 131 ancestral genes potentially lost by pseudogenization by *X. fastidiosa* and *X. albilineans*, respectively (Additional files 2 and 3).

**Common genomic features of *X. fastidiosa* and *X. albilineans***

The close relationship between *X. albilineans* and *X. fastidiosa* is illustrated by the common unique characteristics of their enzymes involved in cellulose degradation. In these two xylem-limited Xanthomonadaceae, endoglucanase EngXCA and 1,4-beta cellobiosidase CbhA possess a cellulose binding domain (CBD) and a long polyserine linker (PSL) at their C termini (Table 2). The endoglucanase EngXCA is conserved in all other Xanthomonas species and also has a CBD, but the linker is much shorter and its serine content is much lower (Table 2). The 1,4-beta cellobiosidase CbhA is conserved in the xylem-invading xanthomonads *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae* but, in these two species, CbhA does not possess any linker nor any CBD (Table 2). The presence of a CBD is known to increase catalytic activity by reducing the "substrate accessibility problem" [22]. The long flexible PSL was proposed to enhance substrate accessibility [23]. The presence of genes encoding enzymes harbouring a PSL and a CBD provides evidence that both *X. fastidiosa* and *X. albilineans* are adapted to use plant cell breakdown products as carbon sources.

The OrthoMCL analyses identified 18 orthologs shared only by *X. fastidiosa* strain 9a5c, *X. fastidiosa* strain Temecula1 and *X. albilineans* GPE PC73 (corresponding to the CDSs conserved in these three strains that are missing in all the other six Xanthomonadaceae genome sequences analysed herein). BLAST analyses confirmed that 11 of these 18 CDSs are unique to *X. albilineans* and *X. fastidiosa* (Additional file 4). Interestingly, they include the gene *metE* which encodes the 5-methyltetrahydropteroyl-triglutamate-homocysteine methyltransferase. This enzyme is absolutely required for the biosynthesis of methionine and is therefore present in all Xanthomonadaceae. However, the *metE* gene present in the two xylem-limited Xanthomonadaceae is closer to the gene *metE* of *Mesorhizobium* sp. (amino acid identity = 466/764 = 60%) than the *metE* present in the other Xanthomonas species and in *S. malophilia* (amino acid identity 89/346 = 25%). This strongly suggests that the progenitor of the two xylem-limited Xanthomonadaceae lost the ancestral *metE* gene (which was conserved in other Xanthomonas species and *S. malophilia*) and acquired another *metE* by horizontal genetic transfer. The 11 genes unique to *X. albilineans* and *X. fastidiosa* also include one cysteine protease gene, one ABC transporter gene, one polysaccharide deacetylses gene, one glycosyl transferase gene, one hydrolase gene, one cell filamentation protein gene and four hypothetical protein genes (Additional file 4).

BLAST analyses confirmed that *X. albilineans*, like *X. fastidiosa*, lacks the Hrp T3SS that is present in other Xanthomonas species and does not possess any of the known Hrp type III effectors. The Hrp T3SS, which plays a major role in suppressing host plant defense responses in most other pathogenic Xanthomonas strains [24], was therefore probably acquired after the divergence of the Hrp Xanthomonas group and xylem-limited Xanthomonadaceae lineages. No remains of the Hrp gene cluster were found in the complete genome sequence of *X. albilineans* strain GPE PC73 nor in the available complete genome sequences of *X. fastidiosa*.

**Discussion**

In their rather cloistered environmental niche inside xylem vessels, *X. albilineans* and *X. fastidiosa* may have largely avoided surveillance by general and specific plant
defense systems. Their lack of a T3SS of the Hrp1 or Hrp2 families may be explained by the fact that X. albilineans and X. fastidiosa live and multiply essentially in a dead-cell environment. However, like other bacterial vascular pathogens, they may interact with living xylem parenchyma cells through pit membranes [25]. If they do, they do not use a Hrp TTSS but another system that remains to be identified. The adaptation of X. albilineans and X. fastidiosa to a xylem-limited lifestyle is also illustrated by their enzymes adapted to the use of plant cell breakdown products as carbon sources. The low number of genes unique to both X. albilineans and X. fastidiosa (11, see Additional file 4) may be explained by a very early divergence of the two xylem-limited Xanthomonadaceae lineages, possibly followed by strong selective pressure to adapt to their different biological niches and lifestyles. X. fastidiosa is vector-transmitted by various xylem sap-feeding insects and is able to colonize many plant species (citrus, wine grape, coffee, alfalfa, peach, plum, almond, elm, maple, pear, etc) (reviewed in [26]). On the other hand, X. albilineans is primarily transmitted by mechanical means and is not known to be insect-transmitted, and is able to colonize only sugarcane and few other monocots in the Poaceae family (reviewed in [8]).

The genome of X. albilineans encodes a T3SS that displays similarities with the Burkholderia pseudomallei bsa T3SS which belongs to the injectisome family SPI-1 (Salmonella Pathogenicity Island -1) and which is required for the virulence of this human pathogen. The SPI-1 injectisome family mainly includes T3SSs from human and insect bacterial pathogens or symbionts [21]. Interestingly, the genomes of Erwinia amylovora strain Ea273 and Erwinia tasmaniensis strain Et1/99 both contain two copies of a SPI-1 T3SS [27,28]. The role of these SPI-1 T3SSs in these plant-invading Erwinia spp. remains unknown. E. amylovora is insect-disseminated, although the interactions between this pathogen and its insect hosts remain poorly understood. It was suggested that the presence of a SPI-1 T3SS in Erwinia spp. indicates a common ancestry and close phylogenetic relationship between Erwinia spp. and insect-related enteric bacteria, raising the possibility that an insect host might be serving as a mixing vessel for the exchange of genes between Erwinia strains and other enteric bacteria [27]. Similarly, the presence of a SPI-1

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**Figure 5**

Distribution in each functional COG category of the putative ancestral genes of X. axonopodis pv.vesicatoria that were conserved or lost by X. albilineans or X. fastidiosa. These putative ancestral genes correspond to the 2816 CDSs of the X. axonopodis pv.vesicatoria strain 85-10 chromosome shared with S. maltophilia strain R551-3, S. maltophilia strain K279a, X. albilineans strain GPE PC73 or X. fastidiosa strain 9a5c. They are listed and individually analysed in Additional file 1. XCV = X. axonopodis pv.vesicatoria strain 85-10; XAL = X. albilineans strain GPE PC73; SMA = S. maltophilia strain R551-3 or S. maltophilia strain K279a; XYL = X. fastidiosa strain 9a5c.
T3SS in the genome of *X. albilineans* could indicate an insect-associated life style of this plant pathogen.

The MLSA performed herein resulted in a phylogenetic tree that included *X. fastidiosa* into the Xanthomonas group. This phylogenetic tree is in accordance with the presence of the unique *gum* genes in both *X. fastidiosa* and Xanthomonas species of the Hrp Xanthomonas group. The *gum* genes, which are involved in the biosynthesis of extracellular polysaccharides and the formation of biofilms, play a key role in pathogenicity of these Xanthomonadaceae. These genes, which were probably acquired by the progenitor of the Xanthomonas genus, were most likely lost by *X. albilineans* and conserved by *X. fastidiosa* during their speciation. Our MLSA phylogenetic tree is also in accordance with i/the presence of 11 unique genes, including *metE*, in *X. albilineans* and *X. fastidiosa*, and ii/the alignment of the 5' end of the 16S RNA of Xanthomonadaceae (Additional file 5).

Additionally, based on this MLSA, the same 480 ancestral genes appeared to be lost by both *X. albilineans* and *X. fastidiosa*. Interestingly, 209 of the 480 ancestral genes lost by both *X. albilineans* and *X. fastidiosa* are also absent in *X. oryzae* pv. *oryzae* (a xylem invading pathogen belonging to another phylogenetic clade), indicating that independent but convergent evolution events were involved in genome erosion of *X. oryzae* pv. *oryzae* and the xylem-limited Xanthomonadaceae. Some of these genes lost by three xylem-invading pathogens are orthologous of genes with assigned functions and are organized into clusters. The five following ancestral gene clusters were lost by *X. albilineans*, *X. fastidiosa* and *X. oryzae* pv. *oryzae*: i/ the ancestral genes XCV0258 to XCV265 encoding enzymes involved in the glyoxylate cycle; ii/ the ancestral genes XCV0592 to XCV0620 encoding enzymes involved in malonate metabolism; iii/ the ancestral genes XCV1316 to XCV1334 including one TonB-dependant receptor gene, a two component signal transduction system (TCSTS) and chemotaxis genes, iv/ the ancestral genes XCV2187 to XCV2196 including one TCSTS and a type I secretion system and v/ the ancestral genes XCV2796 to XCV2803 encoding enzymes involved in catabolism of polysaccharides (Additional file 1). These examples support the hypothesis of a link between the convergent erosion of three xylem-invading Xanthomonadaceae and the adaptation to a same restricted environment (the xylem) in which these lost functions are useless. However, only 38 of the 480 ancestral genes lost by both *X. albilineans* and *X. fastidiosa* are also absent in another xylem invading pathogen, *X. campestris* pv. *campestris*, indicating that adaptation to xylem lifestyle favoured or allowed genome erosion, but did not necessarily induce it. Alternatively, the convergent genome erosion of the two xylem-limited Xanthomonadaceae may be linked to similar insect-associated lifestyles that may have favoured genome erosion because most of the genes required for a plant-associated life style are most likely not required for an insect-associated life style.

Similar striking convergence in fundamental genomic features associated with a restricted lifestyle is very well documented for obligate animal symbionts and pathogens, especially for *Buchnera* (reviewed in [29]). In these bacteria, gene losses are non-random but can affect all functional categories. The most dramatic losses affect genes that are involved in metabolism but are not required for survival. Another general feature is the loss of most DNA repair systems and transcriptional regulatory mecha-
nisms, indicating that there is reduced need for transcriptional regulation in a stable environment [29]. In X. fastidiosa, and to a lesser extent in X. albilineans, losses also affected genes involved in metabolism and transcriptional regulatory mechanisms (Figure 5). Metabolic capabilities essential for other habitats may have been lost in the genome reduction process coincidently with the adaptation of X. fastidiosa and X. albilineans to the nutrient-poor xylem environment. For X. fastidiosa, genome erosion has been extreme. For example, X. fastidiosa retained only one transcriptional sigma factor gene and one outer membrane efflux protein tolC gene, and it lost all genes involved in synthesis of the flagellar apparatus. This extreme erosion allowed X. fastidiosa to save energy (synthesis and operation of the flagella confer a growth disadvantage of about 2% [30]).

In obligate animal symbionts and pathogens, the process of genome shrinkage might have taken place in two separate stages [29]. A massive gene loss must have occurred soon after the establishment of the obligate symbiosis, probably by means of large deletions that eliminated a series of contiguous genes. The large DNA region containing the flagellar genes was probably lost by X. fastidiosa during a similar stage. The accumulation of mobile elements, representing a source of chromosomal rearrangements and gene inactivation, seems to have an important role in this first stage. A similar process is likely responsible for the limited genome erosion of X. oryzae pv. oryzae, which possesses a very high number of insertion sequences (IS) covering 20% of the genome [31]. During the second stage of genome reduction in obligate animal symbionts and pathogens, genome shrinkage seems to have mostly occurred through a process of gradual gene loss, scattered along the genome. Such losses seem to follow a pattern that starts with the inactivation of a gene (pseudogenization) by single-nucleotide mutations, and continues with a rapid reduction in length until the original gene is completely eroded [29,32]. A similar process is likely responsible for the genome erosions of X. fastidiosa and X. albilineans (Additional files 2 and 3). Furthermore, the coding density of X. fastidiosa strain Temecula1 is significantly smaller than that of xanthomonads probably because of the degradation of ancestral genes. In X. fastidiosa strain 9a5c, the number of short annotated CDSs is considerably higher than in other Xanthomonadaceae (Table 1), although the functionality of these shortened CDSs, which may result from the degradation of ancestral genes, is questionable.

Obligate animal symbionts and pathogens display rapid evolution and have highly biased nucleotide base compositions with elevated frequencies of adenine and thymine (A+T) [reviewed in [29]]. X. fastidiosa also displays rapid evolution (note that the length of the branch separating X. fastidiosa from the ancestor common to X. albilineans and X. fastidiosa is much longer, Figure 3) and has a high A+T content in comparison with other Xanthomonadaceae (Table 1). Furthermore, the GC skew pattern of the chromosome of X. fastidiosa has very high amplitude and contains a high number of diagram distortions (Figure 2). A similar atypical GC skew pattern was observed for the chromosome of a Buchnera aphidicola strain [33]. This latter atypical GC skew coincides with the loss of genes involved in the replication restart process (recA and priA) and may be explained by a higher frequency of cytosine deaminations [34]. The loss of DNA repair genes recX, dinG and dinP may explain, similarly, the very high GC skew of X. fastidiosa. It may also explain the more extensive genome erosion of X. fastidiosa, compared to X. albilineans and X. oryzae pv. oryzae. Alternatively, the most important factor affecting genome erosion of X. fastidiosa may reflect the insect-associated lifestyle specific to this Xanthomonadaceae [26].

The GC skew pattern of the X. albilineans chromosome contains a lower number of distortions and has a significantly higher amplitude than the GC skew pattern of other Xanthomonas species (Figure 2), indicating that no recent events of recombination have occurred in X. albilineans. Furthermore, the synteny between the chromosomes of X. albilineans strain GPE PC73 and X. axonopodis pv. vesicatoria strain 85-10 also indicated that recombination events were limited during the speciation of X. albilineans (Additional file 1). The limited recombination of the chromosome of X. albilineans, its limited erosion, its high G+C content and its low number of IS elements may indicate that a distinctive process was responsible for the reductive genome evolution of this pathogen.

We propose a unique mechanism of genome erosion involving the unique toxin albidicin produced by X. albilineans. Albidicin is a potent DNA gyrase inhibitor with 50% inhibitory concentrations (40 to 50 nM) lower than those of most quinolones [9]. DNA gyrase inhibitors block the religation of cleaved DNA intermediate during gyrase catalysis, resulting in lethal double-stranded DNA breaks [9,35]. In the presence of subinhibitory doses of DNA gyrase inhibitors, the SOS response mediates survival of the bacteria by allowing DNA replication to continue past breaks that would normally block it. In exchange for this survival advantage, there is an increased mutation rate because the polymerases that perform the repair are prone to error [36,37]. Several studies showed that subinhibitory doses of quinolones result in an increased mutation rate in Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Mycobacterium tuberculosis [35,38-40]. X. albilineans has two genes conferring resistance to albidicin: an albidicin efflux pump gene that is present in the albidicin biosynthesis gene cluster XALB1 [20,41] and an albidicin-resistant DNA gyrase A gene elsewhere on the chromosome. This albidicin-resistant DNA
Gyrase A is unique to X. albilineans [42]. It contains a unique insertion of 43 amino-acids length close to the albicidin binding site. Production of albicidin in ancestral bacteria that possessed both the albicidin biosynthesis gene cluster and a DNA gyrase A sensitive to albicidin may have induced genome erosion. In these ancestral bacteria, most of the albicidin molecules were secreted by the albicidin efflux pump. Occasionally, molecules of albicidin that were not secreted most likely had the same effect as subinhibitory doses of quinolones: the SOS response was induced, thus resulting in DNA repair, recombination and mutagenesis. Successive and cumulative effect of albicidin at each replication cycle eventually resulted in genome erosion. The genome erosion induced by albicidin was likely arrested by evolution of the albicidin-resistant DNA gyrase A.

Acquisition of the albicidin biosynthesis genes by the ancestor of X. albilineans conferred a selective advantage because of the potent antibiotic activity of albicidin. The DNA damage caused by albicidin may rapidly have induced the mutation of DNA gyrase A gene and thus stopped the process of genome erosion, possibly explaining the distinctive genomic characteristics of X. albilineans. Albicidin inhibits the growth of X. axonopodis pv. vesicatoria (data not shown), suggesting that the DNA gyrase A of the ancestral Xanthomonas was sensitive to albicidin. Transfer of the albicidin biosynthesis gene cluster to X. axonopodis pv. vesicatoria led to production of functional albicidin [43], demonstrating that the production of albicidin per se is not lethal for a producer that possesses an albicidin-sensitive DNA gyrase A. No remains of the albicidin biosynthesis genes were found in the complete genome sequences of X. fastidiosa. Therefore, albicidin is most likely not responsible for genome erosion of X. fastidiosa. However, we cannot exclude the hypothesis that albicidin biosynthesis genes were lost during evolution of X. fastidiosa. For example, cluster XALB1 could have been lost concurrently with the flagellar biosynthesis gene cluster because these two gene clusters are close on the chromosome of X. albilineans.

Conclusions
During their descent from a common ancestral parent, the two xylem-limited Xanthomonadaceae experienced a convergent reductive evolution. Adaptation to the nutrient-poor xylem elements and to the cloistered environmental niche of xylem vessels probably favoured this convergent evolution. Alternatively, the most important factor affecting genome erosion of X. fastidiosa and X. albilineans may reflect insect-associated lifestyles specific to these Xanthomonadaceae. X. albilineans and X. fastidiosa evolved differently: genome erosion has occurred to different extents and specific genes have been acquired independently by X. albilineans and X. fastidiosa. For example, X. albilineans has acquired a T3SS of the SPI-1 family that is mainly found in free-living animal pathogens and four NRPS gene clusters that are involved in the biosynthesis of albicidin and probably other unknown small molecules. The toxin albicidin may be responsible for the distinctive genome erosion of X. albilineans. Much progress has been recently made in understanding how X. fastidiosa spreads within the xylem vessels as well as the traits that contribute to its acquisition and transmission by sharpshooter vectors (For review, [26]). A similar in-depth functional analysis will be necessary to identify the genes that are required for X. albilineans to spread and succeed within sugarcane xylem vessels.

Methods
Bacterial strain
X. albilineans strain GPE PC73 was isolated from a diseased stalk of sugarcane cv. H63-1418 in Guadeloupe (France, [11]). Sequenced strain GPE PC73 is referred to as CFBP 7063 in the French Collection of Plant Pathogenic Bacteria ([44]http://www.angers.inra.fr/cfbp/).

Genome sequencing, assembly and finishing
The complete genome sequence of X. albilineans was determined using the whole-genome shotgun method. Three libraries (A, B, and C) were constructed; two of them were obtained after mechanical shearing of genomic DNA and cloning of generated 3 Kbp and 10 Kbp inserts into plasmids pcdna2.1 (Invitrogen) (A) and pCNS (B) (pSU18 derived), respectively. Larger DNA fragments of about 25 Kbp (generated after partial digestion with Sau3A) were introduced into plasmid pBlO Bac11 to generate a BAC library (C). Plasmid DNAs were purified and end-sequenced (33792 clones for A, 10752 for B and 4800 for C) by dye-terminator chemistry with ABI3730 sequencers (Applied Biosystems, Foster City, USA) leading to an approximately 17-fold coverage. The Phred/Phrap/Consed software package ([45]http://www.phrap.com) was used for sequence assembly and quality assessment. A total of 2151 additional sequence reactions were necessary for gap closure and sequence polishing that consisted of random sequencing of subclones (for 1625 sequence reactions) supplemented with 145 sequences of PCR-products and 381 sequences of oligonucleotide-targeted regions. Final error estimation rate as computed by phred/phrap/consed was less than 0.04 errors per 10 Kbp. The sequences reported here have been deposited in the EMBL GenBank database, and accession numbers are FP565176, FP340279, FP340278 and FP340277 for the chromosome and for plasmids plasmI, plasmII and plasmIII, respectively.

Gene prediction and annotation
Sequence analysis and annotation were performed using iANT (integrated ANnotation Tool; [46]) as described for R. solanacearum [47]. The probabilistic Markov model for coding regions used by the gene prediction software
FrameD [48] was constructed with a set of CDS sequences obtained from the public databank Swiss-Prot as revealed by BLASTX analysis. The alternative matrices were built using genes first identified in ACURs (Alternative Codon Usage Regions) based on homology and taken from the R. solanacearum annotation process [47]. Predicted CDSs were reviewed individually by gene annotators for start codon assignment. The corresponding products were automatically annotated using a protocol based on HAMAP scan [49], InterPro domain annotation and BLASTP analysis. Results were individually expertized to generate the proposed annotations. Proteins were classified according to MultiFun classification [50]. The complete annotated genetic map, search tools (SRS, BLAST), annotation and process classification are available at http://iant.toulouse.inra.fr/X.albilineans [51].

**Phylogenetic analysis**

A phylogenetic tree was constructed from MLSA, with the maximum likelihood method and GTR as substitution model (with I: 0.01 and G: 0.52). The seven loci chosen, gyrB, groEL, recA, dnaK, efp, atpD and glnA, are typically selected housekeeping genes located at the following positions of the X. albilineans chromosome: 0.004, 0.348, 1.369, 1.983, 2.245, 3.442, and 3.655 Mb from the origin of replication, respectively. The total length of the concatenated group of full length CDSs nucleotide sequences was 10417 bp-10686 bp. The tree obtained with the concatenated data set of the seven housekeeping genes was constructed with B. pseudomallei strain NCTC 10247 as outgroup. Multiple alignments of the nucleotide sequences of the 7 housekeeping genes (gyrB, atpD, dnaK, efp, groEL, glnA, recA) for the 11 taxons were performed using ClustalW (The nucleotide alignment is provided in Additional file 6). The phylogenetic tree was calculated with PHYLML ([52,53]; http://atgc.lirmm.fr/phyml/; version 2.4.4).

**OrthoMCL analysis**

OrthoMCL clustering analyses were performed using the following parameters: P-value Cut-off = 1 × 10^-5; Percent Identity Cut-off = 0; Percent Match Cut-off = 80; MCL Inflation = 1.5; Maximum Weight = 316. We modified OrthoMCL analysis by inactivating the filter query sequence during the BLASTP pre-process. All CDSs of X. axonopodis pv. vesicatoria strain 85-10 identified by OrthoMCL and Best hit BLAST analyses as orthologs of two ancestral genes that are contiguous and conserved in X. fastidiosa, S. maltophilia strain R551-3 or S. maltophilia strain K279a. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2164-10-616-S2.xls]

**Abbreviations**

ACURs: alternative codon usage regions; CBD: cellulose binding domain; CDSs: protein-coding sequences; Hrp: hypersensitive response and pathogenicity; IS: insertion sequences; MLSA: multilocus sequence analysing; NRPSs: nonribosomal peptide synthetases; PSL: long polylserine linker; rpf: regulation of pathogenicity factors; SPI-1: Salmonella pathogenicity island -1; T3SS: Type III secretion system; TCSTS: two component signal transduction system.

**Authors’ contributions**

IP and MR contributed to manual annotation of the genome, analysed the data, drafted part of the manuscript and coordinated the project. VB, AC, SM, BS (Segurens) performed sequencing of the genome. SC (Carrere) and JG performed automatic annotation of the genome and OrthoMCL analysis. RK and SC (Cociancich) contributed to manual annotation of the genome and drafted part of the manuscript. CM, VV and MA conceived the study and revised the manuscript. AD, M-A J, EI, SP, BS (Szurke) contributed to manual annotation of the genome and revised the manuscript. PR conceived the study, contributed to manual annotation of the genome and drafted part of the manuscript. All authors read and approved the final manuscript.

**Additional material**

**Additional file 1**

List and individual analysis of the 2816 ancestral genes identified in the genome of X. axonopodis pv. vesicatoria strain 85-10. List and individual analysis of the 2816 CDSs of X. axonopodis pv. vesicatoria strain 85-10 identified by OrthoMCL and Best hit BLAST analyses as conserved in X. albilineans strain GPE PC73, X. fastidiosa strain 9a5c, S. maltophilia strain R551-3 or S. maltophilia strain K279a. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2164-10-616-S1.xls]

**Additional file 2**

List of ancestral genes potentially lost by pseudogenization and short deletions in X. fastidiosa. Analysis of lost ancestral genes that are present on the chromosome of X. axonopodis pv. vesicatoria between the orthologs of two ancestral genes that are contiguous and conserved in X. fastidiosa. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2164-10-616-S2.xls]

**Additional file 3**

List of ancestral genes potentially lost by pseudogenization and short deletions in X. albilineans. Analysis of lost ancestral genes that are present on the chromosome of X. axonopodis pv. vesicatoria between the orthologs of two ancestral genes that are contiguous and conserved in X. albilineans. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2164-10-616-S3.xls]
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