A network approach to decipher the dynamics of Lysobacteraceae plasmid gene sharing

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Received: 12 October 2021 | Revised: 21 April 2022 | Accepted: 5 May 2022

DOI: 10.1111/mec.16536

SPECIAL ISSUE

MOLECULAR ECOLOGY WILEY

A network approach to decipher the dynamics of Lysobacteraceae plasmid gene sharing

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Funding information
The European Union (ERDF contract GURDT I2016-1731-0006632), Conseil Régional de La Réunion, ANSES and CIRAD provided financial support. This work was supported by Agropolis Fondation (Labex Agro – Montpellier, E-SPACE project no. 1504-004).

Handling Editor: Tatiana Giraud

Abstract

Plasmids provide an efficient vehicle for gene sharing among bacterial populations, playing a key role in bacterial evolution. Network approaches are particularly suitable to represent multipartite relationships and are useful tools to characterize plasmid-mediated gene sharing events. The bacterial family Lysobacteraceae includes plant commensal, plant pathogenic and opportunistic human pathogens for which plasmid-mediated adaptation has been reported. We searched for homologues of plasmid gene sequences from this family in the entire diversity of available bacterial genome sequences and built a network of plasmid gene sharing from the results. While plasmid genes are openly shared between the bacteria of the family Lysobacteraceae, taxonomy strongly defined the boundaries of these exchanges, which only barely reached other families. Most inferred plasmid gene sharing events involved a few genes only, and evidence of full plasmid transfers were restricted to taxonomically closely related taxa. We detected multiple plasmid–chromosome gene transfers, including the known sharing of a heavy metal resistance transposon. In the network, bacterial lifestyles shaped substructures of isolates colonizing specific ecological niches and harbouring specific types of resistance genes. Genes associated with pathogenicity or antibiotic and metal resistance were among those that most importantly structured the network, highlighting the imprints of human-mediated selective pressure on pathogenic populations. A massive sequencing effort on environmental Lysobacteraceae is therefore required to refine our understanding of how this reservoir fuels the emergence and the spread of genes among this family and its potential impact on plant, animal and human health.

KEYWORDS
horizontal gene transfer, Lysobacteraceae, network of gene sharing, plasmid, *Xanthomonas*

1 | INTRODUCTION

One of the most striking revelations after two decades of whole bacterial genome sequencing is the great variability in gene content that closely related bacteria can exhibit (Baltrus et al., 2017; Cordero & Polz, 2014; Hyun et al., 2012; Shapiro et al., 2012; Tenaillon et al., 2010). Seminal work on genome content evolution soon indicated that these variations can arise at an unexpectedly rapid pace relative to mutations (Ochman et al., 2000). Later, more holistic studies extended this discovery to the whole bacterial history and clearly demonstrated that a great part of bacterial genomes has been acquired by horizontal gene transfer (Dagan et al., 2008;

Wiedenbeck & Cohan, 2011) to such an extent that it was paramount in several speciation events (De la Cruz & Davies, 2000; Gogarten & Townsend, 2005; Ochman et al., 2000). Although many changes in bacterial gene content were found to be neutral or detrimental, horizontal gene transfer allows rapid adaptation to environmental changes, such as the acquisition of biodegradation pathways or determinants of resistance or virulence (De la Cruz & Davies, 2000; Haegeman & Weitz, 2012; Hao & Golding, 2006; Thomas & Nielsen, 2005).

Four mechanisms of horizontal gene transfer between bacteria have been identified to date: transformation, conjugation, transduction and transfer through gene transfer agents (Lang et al., 2017; Thomas & Nielsen, 2005). While a great body of work has been devoted to understanding the functioning of the genetic machinery involved in each process (Hynes et al., 2016; Popa & Dagan, 2011; Seitz & Blokesch, 2013; Smillie et al., 2010; Touchon et al., 2017), the factors that delineate the transfer community are still to be fully understood. Most notably, whereas there is evidence that characteristics such as taxonomy, genome GC content, codon usage or ecological niche might restrict gene sharing between isolates, the relative importance of these barriers remain the subject of intense debate (McInerney, 2013; Polz et al., 2013; Popa et al., 2011; Popa & Dagan, 2011; Skippington & Ragan, 2012).

Whereas some bacterial genera are prone to acquire genes mostly through phage integration (i.e., transduction, such as in *Streptococcus*) or transformation (Dorer et al., 2010), in other taxa, including the genus *Xanthomonas*, horizontal gene transfer mainly occurs through plasmid exchange (Halary et al., 2010). The intercellular mobility of some plasmids (through conjugation) and their capacity to transmit ready-to-use functional pathways is often suggested as the cause of their ubiquity and their central role in bacterial evolution (Che et al., 2021; Corel et al., 2016; Halary et al., 2010; Popa & Dagan, 2011; Rodríguez-Beltrán et al., 2021; Tamminen et al., 2011).

The Gram-negative family Lysobacteraceae (heterotypic synonym Xanthomonadaceae) belongs to the class Gammaproteobacteria and constitutes an important group of plant pathogens, opportunistic human pathogens responsible for nosocomial infections, and commensal bacteria colonizing plants and soil. Within this family, *Xanthomonas* is the most extensively characterized genus, comprising numerous plant pathogenic species that collectively cause diseases on a wide range of crops in agro-ecosystems (Leyns et al., 1984). *Xanthomonas* species typically gather several pathologically highly specialized lineages (i.e., the so-called pathovars) (Garita-Cambronero et al., 2016; Nakato et al., 2018; Pruvost et al., 2014). In the genus *Xanthomonas*, genes including essential virulence genes (Chen et al., 2018; Yang, 1994), antibiotics (Hyun et al., 2012; Minsavage et al., 1990) or metal resistance genes (Niu et al., 2015; Richard et al., 2017) were reported from plasmids and are likely to have played a key role in its evolution. Several other members of this family are also known to contain plasmid-borne drug resistance genes that have been characterized from *Stenotrophomonas maltophilia*, an opportunistic pathogen associated with nosocomial human infections (Avison et al., 2001; Kanamori et al., 2015) and reported as a major reservoir for antimicrobial compound resistance genes (Ryan et al., 2009; Berg & Martinez, 2015). Similarly, *Xylella fastidiosa*, an economically major insect-vectored pathogen (Sicard et al., 2018), also hosts plasmids, at least one of which was described to carry genes involved in resistance to toxic compounds (Rogers & Stenger, 2012).

Using comparative genomics, a previous analysis disentangled the molecular basis of copper resistance (i.e., an antimicrobial widely used worldwide against bacterial and fungal plant pathogens) in *Xanthomonas citri* pv. *citri* (Richard et al., 2017). Specifically, this study evidenced several plasmid-borne heavy metal resistance gene clusters, also detected in other species of the family, either integrated in a highly homologous or distinct plasmid backbone (*Xanthomonas* spp.) or present in the chromosome (*Stenotrophomonas* spp.) (Richard et al., 2017). Besides confirming the high prevalence of plasmids within the family, these findings suggested that the sharing of plasmid genes was not restricted to full-length plasmid exchange. To define whether this finding could be extended to the Lysobacteraceae overall plasmid population and to identify the barriers mostly structuring the plasmid transfer patterns, we analysed the sharing of homologous plasmid genes among the whole set of bacterial genomic sequences available on NCBI databases using a gene-sharing network analysis. After (i) having estimated to what extent plasmids are conserved and shared within the family, we (ii) assessed the influence of the ecological niche and the taxonomy over the patterns of gene sharing and (iii) detected events of gene exchange between plasmids and the chromosome.

2 | MATERIALS AND METHODS

2.1 | Plasmid reference sequences

The complete set of plasmid sequences of the family Lysobacteraceae (sensu [Naushad et al., 2015]) were downloaded on November 2017 from the NCBI nonredundant (NR) database ($n = 441$, obtained from 160 distinct bacterial isolates; i.e., NCBI biosamples). The family contains 17 genera, but complete plasmids were only available for three of them (*Stenotrophomonas*, *Xanthomonas* and *Xylella*). PlasForest did not identify contigs of plasmid origin among sequences available for other Lysobacteraceae genera. For suspicion of being of chromosome rather than plasmid origin, contigs annotated as plasmids but coding for genes typically chromosome-borne (e.g., ribosomal protein genes) were discarded, as well as sequences that were not assembled as circular molecules. From the remaining 305 contigs (obtained from 140 bacterial isolates; Table S1), a total of 19,921 genes were predicted using PRODIGAL version 2.6.2 (Hyatt et al., 2010) with default parameters. These genes were then clustered into 18,715 groups of 100% identical sequences using the derep_fulllength algorithm available in VSEARCH version 1.9.5 (Rognes et al., 2016).

2.2 | Search of homologous sequences in NCBI

We then searched for close homologues of our query plasmid genes in all available NCBI bacterial genomes (Figure 1). To do so, the

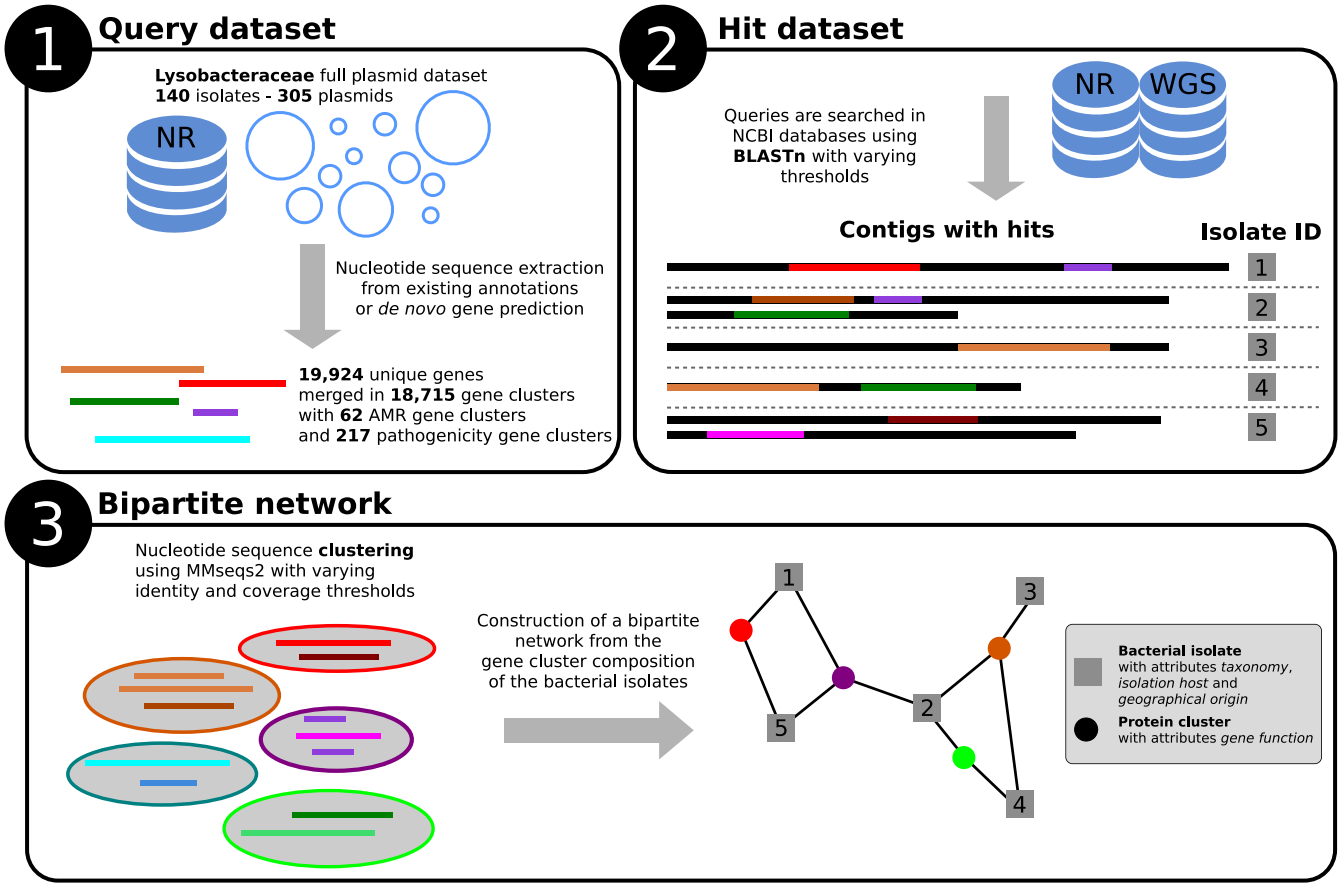

FIGURE 1 Schematic representation of the global methodology for gene sharing network reconstruction. (1) from NCBI NR, all full Lysobacteraceae plasmids were obtained and gene sequences extracted. (2) plasmid gene sequences were searched against the entire NR and WGS databases. (3) hits were then clustered and bipartite networks were built based on the sharing of gene clusters between bacterial isolates. AMR, antimicrobial resistance

18,715 unique gene sequences were used as a query for a BLASTN search against the NR and Whole Genome Shotgun (WGS) databases (45,016 and 214,443 biosamples as of November 2017, respectively) using default parameters except for max_target_seqs that was set to 10,000,000. To evaluate the robustness of the gene sharing network to the similarity threshold used, we constituted subsets of the BLASTN hit table with different levels of stringency for the minimum identity (%id) and the length percentage of the matched query gene (%len). Six combinations (%id/%len) of these two filters were used: 90/90, 95/90, 95/95, 98/90, 98/98 and 99.8/90. For each parameter combination, all NR and WGS contigs matching at least one gene with the minimum identity and coverage defined above were downloaded. Contigs were then classified as plasmid or chromosomal contigs using PLASFOREST (Pradier et al., 2021) with default parameters.

Genes were extracted based on NCBI gene annotation when available or conversely after structural annotation using PRODIGAL version 2.6.2 as described above. Including the query genes, a total of 87,421 genes from 14,576 contigs and 10,112 biosamples were obtained (Table S2). Taxonomies, host plants and geographical origins of all phytopathogenic *Xanthomonas* isolates studied herein were extracted from NCBI metadata and manually curated. Along with the available NCBI functional annotations, all these genes were functionally annotated using a BLASTP search against the Swiss-Prot and Trembl databases (O'Donovan et al., 2002), the Pathogen Host Interaction database using the available online tools (PHI-base; Urban et al., 2019) and the CARD database using the resistance gene identifier tool (Alcock et al., 2019). Annotations were then manually curated and merged. To make manual curation possible given the very large number of genes that were included, we reduced the functional annotations to five categories most relevant to the plasmid nature of the genes and the plant pathogenic nature of the bacterial isolates we focused on: conjugation apparatus, plasmid stabilization, pathogenicity, antibiotics and heavy metal resistance.

2.3 | Delineation of gene clusters

The whole set of gene sequences along with plasmid query genes were then clustered using the "linclust" algorithm of MMSEQS2 (Steinegger & Soding, 2017). Coverage (c parameter) and the minimum identity (min-seq-id parameter) were both set according to values of the corresponding BLASTN filters (%id and %len) previously described. Genes that were assigned to the same cluster were considered homologous. Gene clusters that did not comprise any plasmid

query gene were removed, resulting in a total of 8197 gene clusters (at >99.8% identity along >90% of query length; the number of gene clusters obtained with other thresholds is detailed in Table S2).

2.4 | Bipartite network of gene sharing

Bipartite networks were then constructed using the R package igraph (Csardi & Nepusz, 2006). Two classes of nodes coexist in such networks: bacterial isolates and gene clusters. Whereas no direct link exists between two nodes of the same class, isolates sharing a gene cluster are connected to this same gene cluster node and conversely a gene cluster is connected to all the isolates in which it was detected. To reduce the redundancy of the data set due to unbalanced sequencing efforts and to limit the inclusion of vertical inheritance rather than horizontal transfer, isolates from the same species and containing the exact same gene clusters were merged. This made our network conservative in that it globally represents the minimum number of plasmid gene movements that must have occurred to lead to the current plasmid gene distribution. Community detection (i.e., groups of nodes more connected between each other than they are to the rest of the network) was performed using the Louvain algorithm (Blondel et al., 2008) as implemented in the igraph R package. Importantly, as the Louvain algorithm is not suitable for bipartite networks, the network was first projected onto the isolates (i.e., only isolates nodes remained and the weight of an edge between two isolates corresponded to the number of gene clusters they share) (Banerjee et al., 2017). Degree of the nodes (i.e., their number of adjacent edges) was computed using the igraph R package. In a network, nodes sharing certain characteristics often tend to be more connected than others. Assortativity, a measure of this homophyly (Newman, 2003), was computed using igraph based on (i) country of origin, (ii) plant host family, and (iii) bacterial genus and species. Significance was then obtained after comparison of the obtained value with the distribution of values of randomized networks (the proportion of permuted values exceeding the observed value is used as a *p*-value) and a Bonferroni correction was applied.

2.5 | Groups of genes shared between plasmids and chromosomes

We then searched for chromosomes carrying multiple genes from our query plasmid genes. We started by identifying contigs of chromosomal origin. Because of the relative unfinished nature of the genomic project available on the WGS and NR databases and to avoid over-estimation of the sequence sizes due to improper scaffolding (e.g., chromosomal scaffolds that would contain plasmid contigs), all sequences were split at every occurrence of long N sequences (indicative of a contig junction). We then searched for instances where more than 10 genes from a given query plasmid were found within a single contig of more than 1000 genes identified as chromosome using PLASFOREST. We restricted our analysis to these events of sharing implying a fair number of genes (here 10) in very large contigs to (i) limit the inclusion of small spurious gene sharing events, (ii) avoid the annotation of plasmid sequences as chromosomal (i.e., these contigs exceed the size of the largest plasmids identified so far in bacteria from the family Lysobacteraceae) and (iii) limit the inclusion of contigs from sequencing projects where contiguity is low and therefore the assembly less reliable. Importantly, a position permutation test was implemented to ensure that these genes were more clustered (i.e., spatially close to each other on the contig) than expected by chance on the hit contig. To this end, for a sharing event of *n* genes, the sum of the distance (in number of genes) of each pair of those *n* genes was compared to that obtained after 100 permutations of their positions. Only events where the clustering *p*-value was less than .1 were considered. The rank of the measured sum of distance among the permutated sum of distance is used as a *p*-value of clustering. This scan allowed the detection of the largest and most conserved intergenomic compartment gene sharing events between chromosomes and plasmids.

3 | RESULTS AND DISCUSSION

3.1 | The Lysobacteraceae plasmid database

The query data set comprised 19,921 genes encoded on 305 plasmid contigs from 140 bacterial isolates (i.e., NCBI biosamples) (Table S1 and Figure S1). Isolates belonged to three genera and 19 species, mostly *Xanthomonas citri* (49%), *Xylella fastidiosa* (15%) and *Xanthomonas campestris* (9%). The remaining 16 species represented 5% or less of the database each. Plasmid size was very variable, with small (<10 kb) to intermediate size plasmids (<100 kb) being the most represented (20% and 73%, respectively). Nevertheless, 22 plasmids larger than 100 kb were present and carried 29% of the analysed gene pool. Isolates carried from one plasmid (53 isolates) to nine plasmids (three *Xa. campestris* isolates) with a mean number of 2.9 plasmids per isolate.

The most common predicted gene functions among the 19,921 query plasmid genes were conjugation ($n = 3054$), transposition ($n = 2355$), plasmid persistence ($n = 1414$) and pathogenicity-related functions ($n = 527$). Conjugative apparatus genes were detected in 59% of the plasmids while 50% hosted a toxin–antitoxin system. These systems were mostly detected on plasmids from intermediate to large size (213 out of 219 conjugative contigs were >10 kb). Our query data set comprised 15 distinct antimicrobial resistance gene clusters that confer resistance to acriflavine, beta-lactam antibiotics, bleomycin or streptomycin and 47 heavy metal-resistance gene clusters that confer resistance to arsenic, cadmium, cobalt, copper, mercury, silver and/or zinc. Although heavy metals other than copper are of little use in agriculture (Hobman & Crossman, 2015), their presence can probably be explained by the frequent colocalization of genetic systems conferring resistance against different metals on a single plasmid (Gullberg et al., 2014; Staehlin et al., 2016). Finally, a total of 92 plasmids from 15 species carried genes associated

with pathogenicity. Among these, 28 plasmids carried transcription activator-like effector (TALE) genes that code for proteins that manipulate the expression of host plant genes to promote bacterial colonization (Boch et al., 2009). Overall, our plasmid gene query database mostly comprised genes associated with (i) intrinsic plasmid functions such as partitioning, conjugation, replication and maintenance, or mobile genetic element functions such as transposition, (ii) resistance against anthropic pesticides (copper, streptomycin) used in agriculture, and (iii) host–pathogen interactions.

3.2 | Plasmid genes are readily shared within the family Lysobacteraceae

Gene sharing relationships between our set of query plasmid genes and the whole of the sequences of the NR and WGS NCBI databases were defined between replicons (plasmids or chromosomes) carrying one or more identical gene clusters (see Methods and Figure 1). First, it must be borne in mind that these results are inherently limited and biased by the properties of the databases. Either database composition biases, database redundancies or the quality of the sequences (degree of completion of the genome) would leave strong imprints in the network. For example, nonpathogenic Lysobacteraceae species are poorly represented in NCBI databases. As such, based on a few genomes, some genera possibly artefactually appear plasmid-free. This being said, and to explore the robustness of the gene sharing patterns, we used different clustering thresholds, from relaxed to more stringent ones. Whereas the network built using the less stringent parameter combination contained more genes and more isolates (Table S2), the general structure of the bipartite networks representing gene sharing relationships remained fully consistent from one threshold to the other: a main connected component (i.e., group of connected nodes) that gathered most of the isolates (from 85.9% to 99.7%) and weakly connected non-Lysobacteraceae isolates (Figures S2–S6). Depending on the clustering threshold used, the global gene-sharing network comprised 9–13 distinct connected components. While the threshold stringency had little effect on the number of Lysobacteraceae isolates included in the network, the number of non-Lysobacteraceae varied markedly, the proportion of Lysobacteraceae isolates ranging from 38% to 53% for the more stringent thresholds combination. For the rest of the study, we only considered the network obtained using the most stringent thresholds: to be included in the network, a gene had to match query genes with 99.8% identity on 90% of its length (Figure 2). In this network, the main connected component gathered 99.7% of the isolates. Most of the *Xy. fastidiosa* isolates formed an independent connected component. The remaining 17 isolates (0.2% of the total network) were allocated into 10 connected components and hosted probable spurious small plasmids of fewer than four genes (the unconnected components on the top of Figure 2).

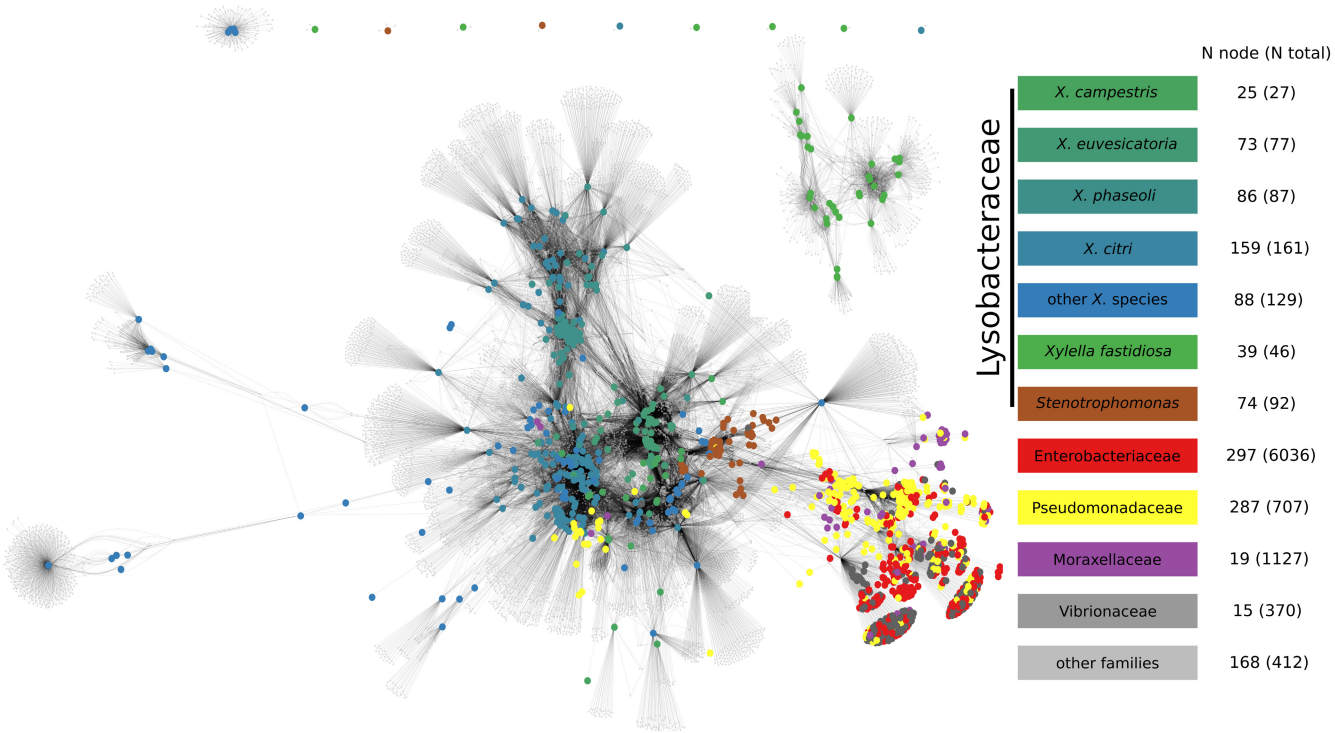

FIGURE 2 Gene sharing bipartite network of the Lysobacteraceae plasmid genes using a 99.8% identity and 90% coverage for gene clustering. Large nodes represent bacterial isolates whereas smaller sized grey nodes represent gene clusters. Bacterial nodes are coloured according to the taxonomy of the isolate they represent. An edge between a bacterial node and a cluster gene node indicates that the bacterial genome carries a gene of this cluster. When multiple isolates of the same bacterial species display the exact same gene cluster content, only one node is represented on the network. For several taxon levels, the number of unique bacterial nodes is given on the right side of the key while the total number of corresponding isolates is in parentheses

Globally, our results show that the Lysobacteraceae plasmid genes are highly shared within the family. Using our most stringent threshold, 9359 isolates coding for 8197 gene clusters were included in the network. To avoid redundancy, the 9359 isolates were merged in 1422 unique combinations of bacterial species and gene content (i.e., isolates of identical species displaying the exact same gene content were merged). Whereas we did not attempt to disentangle vertical and horizontal inheritance of genes, the merging of isolates from the same species and comprising the same gene clusters should mitigate the influence of vertical inheritance on the network. Gene sharing events depicted here should therefore mostly correspond to horizontal gene transfer. Of the 6221 hit contigs, 63% (mean size of 319 genes) were identified as chromosomes and 37% (mean size of 21 genes) were identified as plasmids by PLASFOREST. While no plasmid gene was shared by all isolates, 3944 (48%) genes originating from 36 plasmids were unique to an isolate. The remaining 4253 gene clusters were found on average in 12 isolates (maximum of 404 isolates).

3.3 | From partial to full plasmid sharing

In the global network, most of the gene sharing events involved partial plasmid segments and a few genes (Figure 3), indicative of plasmid mosaicism, a common feature that has been documented in the genus *Xanthomonas* (Gochez et al., 2018; Richard et al., 2017). Several of the Lysobacteraceae plasmids in our database were apparent chimeras relative to each other (see the module over the diagonal on Figure S1b).

Only a fraction of the isolates shared full or almost full (>90%) plasmids. To visualize plasmid sharing rather than gene sharing, networks representing the sharing of almost full plasmids were computed (Figure 4). Importantly, whereas the existence of a link implied the sharing of >90% of the plasmid genes, we had no indication that they were actually carried on a single plasmid or if a similar gene content was borne on multiple plasmids. Whereas several bacterial families were included in the global gene sharing network, only isolates of the family Lysobacteraceae remained in the full plasmid sharing network. This network was composed of 97 connected components, only two of which contained more than 10 isolates. The first connected component (CC#1 on Figure 4) included 63 isolates and 68 plasmids belonging to *Xa. euvesicatoria* and *Xa. campestris* pv. *campestris* (which infect Solanaceae and Brassicaceae plant families, respectively; Leyns et al., 1984). As direct contact between bacteria from both pathovars should be rare, it may require an intermediate bacterial species for horizontal gene transfer to occur. *Xa. campestris* pv. *raphani* could have played this role as it possesses the uncommon feature of being pathogenic to both Solanaceae and Brassicaceae plant families (Fargier & Manceau, 2007; Punina et al., 2007). However, the only two *Xa. campestris* pv. *raphani* genomes in the data set appeared to be plasmid-free. More genome data are therefore required to address this hypothesis (Roux et al., 2015). The second connected component (CC#2 on Figure 4) comprised 12 isolates of *Xa. citri* pv. *citri* which shared plasmids pXCAW19 and pXCAW58. This corresponds to known plasmid horizontal transfer between *Xa. citri* pv. *citri* pathotypes (Gordon et al., 2015). Those results indicate that full plasmid sharing is rare and does not occur between distantly related species. As plasmids are frequently transferred from one bacterium to another as a whole through the conjugation process, our results would imply that other processes are at play for most of the gene transfer beyond the family level.

3.4 | Taxonomy shapes plasmid gene transfer

The network was built using plasmid genes from the family Lysobacteraceae as queries. It therefore delineated the extent to which close homologues of the plasmid genes from this family are present within other bacterial taxa. Our approach retrieved 41% of the Lysobacteraceae and 84% of the *Xanthomonas* genomes available in the WGS and NR databases from NCBI (see Table S2 for details). Indeed, 39% of the isolates of the network belonged to the family Lysobacteraceae. Conversely, beside the absence of full plasmid sharing events beyond the family Lysobacteraceae, inspection of the full bipartite network (Figure 2) demonstrated that interfamily gene sharing was rare and restricted to a small number of genes. In a *k*-mer-based plasmid network study, *Xanthomonas* also appeared as an isolated genus (Acman et al., 2020). Gene sharing events between isolates assigned to distinct bacterial families comprised a mean number of 2.4 genes (against 19.6 for intrafamily events). Most (96.3%) non-Lysobacteraceae isolates were grouped in a sole Louvain community. Non-Lysobacteraceae families displaying the highest proportion of isolates included in the network were Moraxellaceae, Vibrionaceae and Enterobacteriaceae (all of which including Lysobacteraceae are Gammaproteobacteria) for which, respectively, 17.6%, 6.5% and 3.7% of the isolates present in the databases were recovered (Table 1). Assortativity measures the level of homophyly of a network based on a chosen characteristic of its nodes. We computed the assortativity of our network based on the taxonomy (down to the species level) of the isolates and obtained a significantly higher coefficient ($p < 10^{-3}$) for the real network compared to 10,000 identical networks with permuted taxonomies, meaning that connected bacterial isolates tend to be taxonomically similar. These results highlight that the Lysobacteraceae plasmid gene pool is mostly restricted to the family but highly shared within it, and that plasmid gene sharing tends to be reduced to closely related bacteria, as previously emphasized (Popa et al., 2011; Tamminen et al., 2011).

3.5 | Bacterial lifestyle: A secondary barrier to horizontal gene transfer?

Interactions between taxonomic, niche and possibly other functional barriers in the delineation of communities were previously suggested (Popa & Dagan, 2011). The present study provides further examples

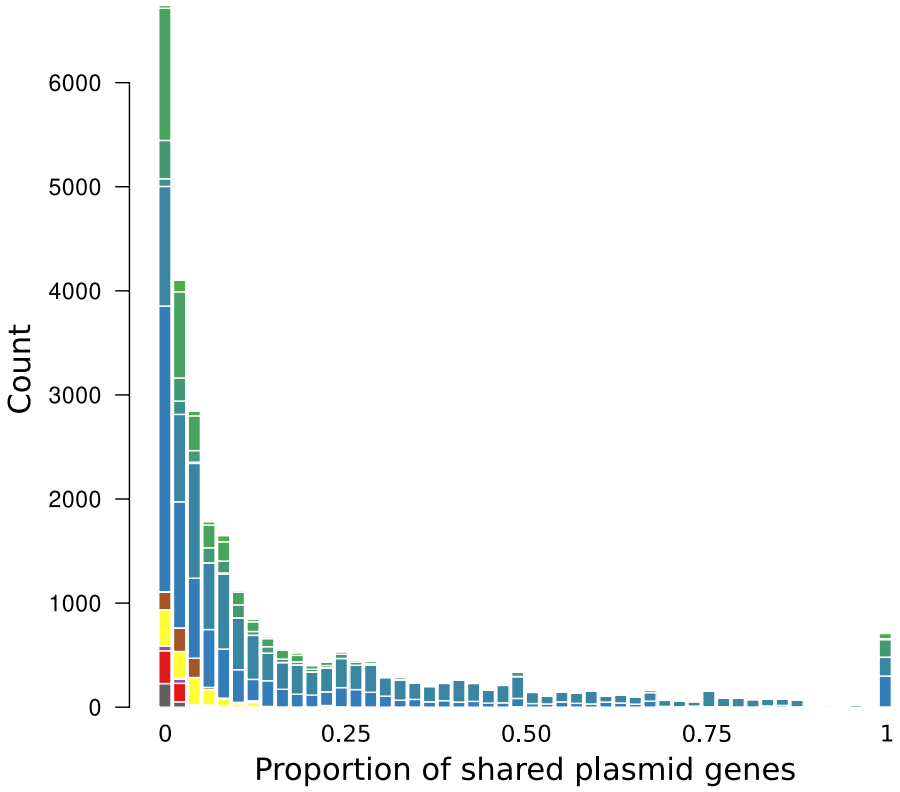

FIGURE 3 Histogram of the percentages of gene clusters of each of the query plasmids found in other bacterial isolates. The percentage (*x*-axis) is computed as the number of gene clusters from a given query plasmid found in a bacterial isolate divided by the total number of gene clusters of this query plasmid. Values are grouped by the taxonomy of the hit bacterial isolate, represented using the colour code of Figure 2

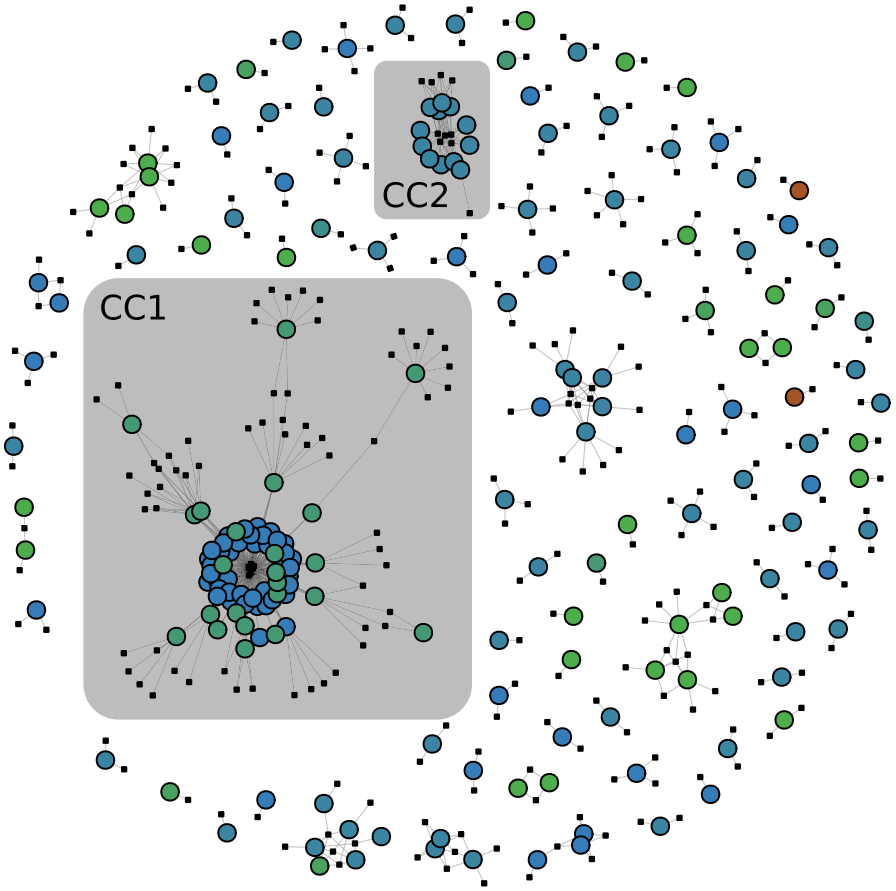

FIGURE 4 Bipartite network of full plasmid sharing. Plasmid nodes are represented by black squares and bacterial nodes are represented by coloured circles. Edges represent events where a bacterial isolate carries at least 90% of the genes of a given plasmid. Colours of bacterial nodes represent the taxonomy of the bacterial isolate as depicted in Figure 2

in the genus *Xanthomonas*. While the network boundaries and structuring in community seemed to be driven by taxonomy, the genus *Xanthomonas* provides a good model to gain insights into the role of bacterial lifestyle in structuring plasmid gene exchange (Figure 5). Indeed, the genus *Xanthomonas*, when taken as a whole, is pathogenic to a large number of monocot and dicot plant species. However, at

the pathovar level, these bacteria often exhibit a high host specialization towards a limited number of genetically related plant species or genera with different tissue specificity (i.e., parenchyma vs. vascular pathogens) (Leyns et al., 1984). In some cases, distinct *Xanthomonas* species can colonize the same host plant niche, a feature known as pathological convergence. The obligatory pathogen nature of *Xanthomonas* makes their host plant a good proxy for their ecological niche. We therefore computed the assortativity (as detailed above) of our network based on the host plants of the bacterial isolates. We again obtained a significantly higher coefficient ($p < 10^{-3}$) for the real network compared to 10,000 identical networks with permuted host plants, meaning that connected bacterial isolates tend to infect the same hosts. However, we must note that the host plant of *Xanthomonas* isolates included in the network is not completely independent of their taxonomy, making the assessment of the most structuring factor difficult. Moreover, all agricultural plant hosts are not cultivated everywhere, making taxon sampling not evenly geographically distributed. As a possible consequence, geography is also a factor significantly structuring the network ($p < 10^{-3}$).

Community #2 of our network grouped *Xa. hortorum* pv. *gardneri*, and *Xa. euvesicatoria* pvs. *Euvesicatoria* and *perforans*, which are pathogenic to tomato and pepper parenchyma and known to have exchanged genes involved in pathogenicity (Jibrin et al., 2018). Similarly, community #1 grouped the two bean pathogens *Xa. phaseoli* pv. *phaseoli* and *Xa. citri* pv. *fuscans*, for which pathological convergence is probably the consequence of the horizontal gene transfer-mediated sharing of plasmids hosting TALE genes (Alavi et al., 2008; Chen et al., 2021). Several pathogenicity-associated genes were shared within the community but did not present a significantly higher degree (i.e., the number of adjacent edges of a node) than other gene functions. However, antimicrobial resistance genes (here, mostly copper and other heavy metal resistance genes) showed a significantly higher degree than other gene functions in this community (Wilcoxon $p < 10^{-3}$, Table S3). This finding is congruent both with the known agricultural practices (copper-based pesticides are frequently used against bacterial infections of tomato and pepper; Osdaghi et al., 2021) and with our previous findings (Richard et al., 2017).

Community #4 groups both commensal (*Stenotrophomonas* spp.) and pathogenic (*Xanthomonas* spp.) bacteria. Commensal *Stenotrophomonas*, often found in association with plants and soil, are suspected to act as a reservoir of resistance genes for human and plant pathogenic bacteria (Crossman et al., 2008). In our network, community #4 might reflect a module of resistance gene sharing. This community comprises 26 distinct antimicrobial resistance genes, which showed a significantly higher degree than other gene functions (Wilcoxon $p < 10^{-4}$, Table S3).

TABLE 1 Number of available biosamples on NCBI NR and WGS per taxonomic group sharing genes with the Lysobacteraceae plasmids at the 99.8% identity and 90% length threshold

Taxonomic level	Taxonomy	No. of biosamples sharing Lysobacteraceae plasmid genes	No. of biosamples in nr and WGS databases	% of NR/WGS biosamples included in the network
Family	Enterobacteriacee	6036	164,587	3.7%
	Vibrionaceae	370	5680	6.5%
	Pseudomonaceae	707	10,810	6.5%
	Moraxellaceae	1127	6386	17.6%
	Lysobacteraceae	621	1506	41.2%
Genus	*Salmonella*	1444	93,616	1.5%
	Burkholderia	93	3611	2.6%
	Escherichia	1793	54,468	3.3%
	Aeromonas	20	501	4.0%
	Pseudomonas	708	10,867	6.5%
	Vibrio	370	5307	7.0%
	Citrobacter	37	512	7.2%
	Serratia	35	402	8.7%
	Proteus	18	202	8.9%
	Shigella	513	5493	9.3%
	Corynebacterium	109	1120	9.7%
	Acinetobacer	1126	6279	17.9%
	Klebsiella	1748	9685	18.0%
	Stenotrophomonas	92	489	18.8%
	Sphingobium	21	102	20.6%
	Enterobacter	484	1946	24.9%
	Xyllela	46	94	48.9%
	Xanthomonas	481	782	61.5%

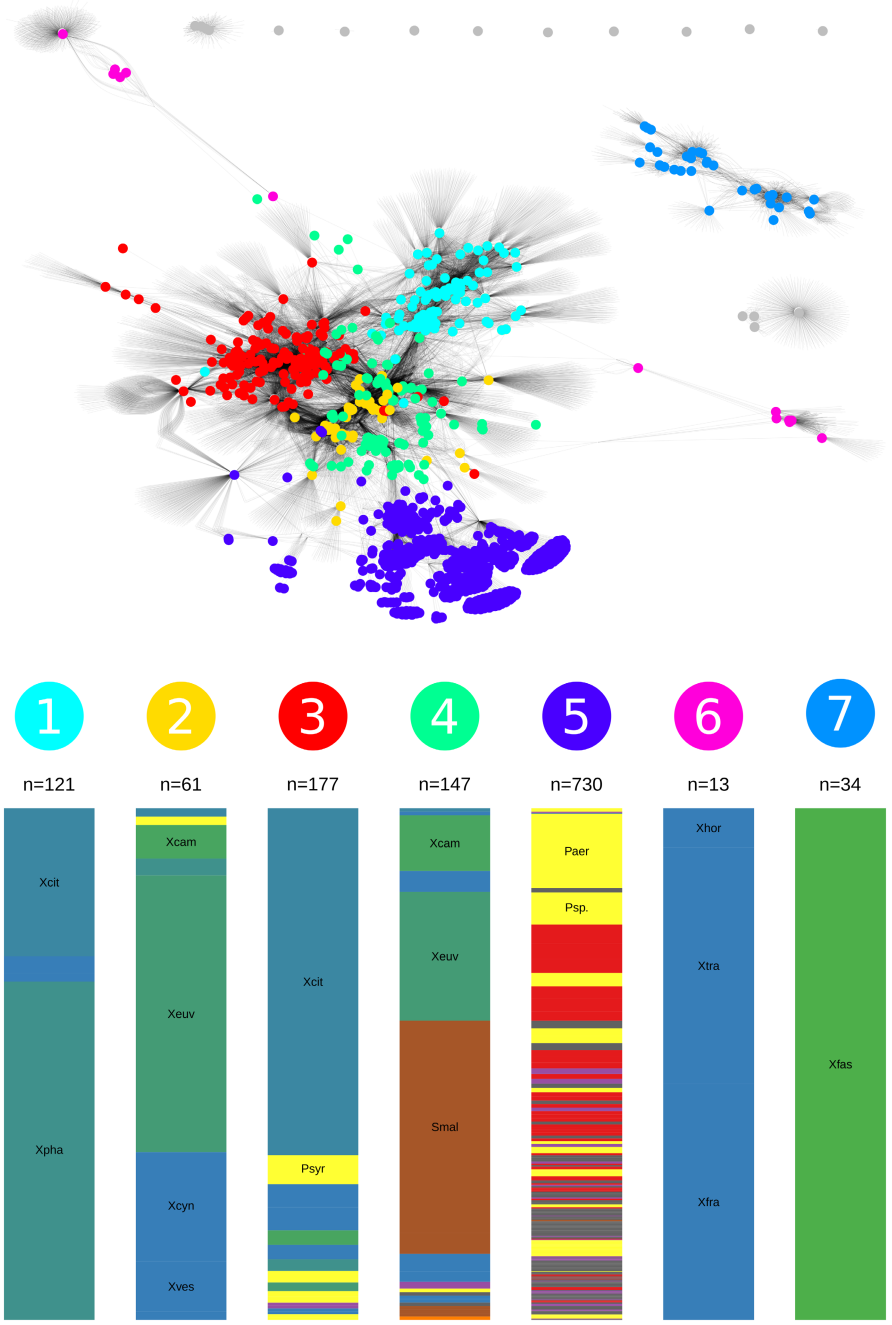

FIGURE 5 Representation of community membership as inferred using the Louvain algorithm over the bipartite network of plasmid gene sharing. Bacterial nodes belonging to the seven main communities are coloured according to their Louvain community membership as shown in the key at the bottom half of the figure. The barplot graph indicates the taxonomy of the bacteria of each community. Numbers over each bar give the number of isolates included in each community. Bars are divided into tracks whose size is proportional to the number of bacteria of each taxon level and are coloured according to Figure 2. For tracks representing more than 5% of a given community, an abbreviation of the bacterial species name is given: Paer, *Pseudomonas aeruginosa*; Psp., *P.* sp.; Psyr, *P. syringae*; Smal, *Stenotrophomonas maltophilia*; Xcam, *Xanthomonas campestris*; Xcit, *Xa. Citri*; Xcyn, *Xa. Cynarae*; Xeuv, *Xa. Euvesicatoria*; Xfra, *Xa. Frageriae*; Xhor, *Xa. Hortorum*; Xpha, *Xa. Phaseoli*; Xtra, *Xa. Translucens*; Xves, *Xa. Vesicatoria*; Xfas, *Xy. Fastidiosa*

The second largest connected component in our gene sharing bipartite network (which perfectly corresponds to community #7) uniquely gathered *Xy. fastidiosa* isolates, illustrating a case of both taxonomy and niche barrier. This taxon experienced substantial genome reduction during evolution and colonized xylem vessels through insect transmission (Huang et al., 2020; Sicard et al., 2018). Although the plant host range of this bacterium is very large, it does not overlap that of *Xanthomonas* colonizing the same plant tissues, strongly restraining the probability that these two bacteria meet. In contrast, *Stenotrophomonas* sequences were identified in the microbiota of the *Xy. fastidiosa*-transmitting glassy-winged sharpshooter (*Homalodisca vitripennis*) as well as in xylem sap of grapevine under high Pierce's disease pressure (Curley et al., 2007; Deyett & Rolshausen, 2019; Hail et al., 2011). Our results did not highlight any plasmid gene sharing between those two genera. Given the striking importance of *Xylella* as a crop pathogen, improving our understanding of horizontal gene transfer between *Xylella* and other bacteria in insects and xylem sap is a challenging area for future research.

3.6 | Multiple gene transfers between plasmids and chromosomes

While plasmids are efficient vehicles for genes conferring a selective advantage in response to a biotic or abiotic selection pressure, plasmid maintenance in hosts is metabolically costly, potentially leading

TABLE 2 Characteristics of the identified gene transfer events involving a plasmid and a chromosome

Inter-replicon gene sharing event ID	Query plasmid taxonomy	Accession of the query plasmid representative sequence[a]	Chromosome hit taxonomy	Maximum number of genes shared[b]	Main function(s) of the shared genes
1	*Xa. citri*/*X. euvesicatoria*	CP018464	*S. maltophilia*/*S.* sp.	38	Heavy metal resistance
2	*Xa. vesicatoria*	CP018471	*P. aeruginosa*/*P. mendocina*/*P. pseudoalcaligenes*/*P. rhodesiae*	11	Mercuric resistance
3	*Xa. euvesicatoria*	CP018472	*Xa. euvesicatoria*	117	Conjugation
4	*Xa. citri*	CM002265	*Xa. campestris*	14	Conjugation
5	*Xa. citri*	CM007623	*Xa. citri*	22	Restriction-modification
6	*Xa. citri*/*Xa. phaseoli*	CP020966	*Xa. citri*/*Xa. phaseoli*	24	Toxin–antitoxin and conjugation
7	*Xy. fastidiosa*	CM003750	*Xy. fastidiosa*	35	Toxin–antitoxin and conjugation
8	*Xa. translucens*	CM003053	*Xa. translucens*	11	Conjugation
9	*Xa. cassavae*/*Xa. citri*/*Xa. oryzae*	AE008925	*Xa. cassavae*/*Xa. citri*/*Xa. oryzae*	33	Avirulence, toxin–antitoxin and conjugation

[a]One of the plasmids from the query set that harbour the genes involved in the event.

[b]Similar events were merged (see Methods). For each group of similar inter-replicon gene sharing events, we report here the maximal number of genes shared.

to the integration of beneficial genes originally carried on plasmids into the bacterial chromosome (Harrison & Brockhurst, 2012). By contrast, the integration of chromosomal genes into a plasmid may facilitate their spread in bacterial communities, as illustrated by the success of the plasmid/transposon association (Bennett, 2004; Davies, 1997; Liebert et al., 1999; Okinaka et al., 1999). The analysis of copper resistance in *Xa. citri* strains highlighted a plasmid-located transposon (Richard et al., 2017). Gene similarity analysis revealed the presence of this very transposon integrated into a distinct plasmid backbone in *Xa. euvesicatoria* but also into the chromosome of a strain of a commensal *Stenotrophomonas* species. We sought, from our data set, to identify such transfers among replicons to evaluate whether it is a common process within the Lysobacteraceae. To do so, we screened large putative chromosomal contigs (>1000 genes, all identified as chromosomes using PLASFOREST) for the presence of gene clusters shared with query plasmids (Figure S7). Despite the implementation of a stringent filtering (i.e., requiring the sharing of at least 10 genes to consider an event, to prevent the detection of insertion sequences and other small ubiquitous genetic elements), the analysis revealed that a minimum of nine distinct inter-replicon gene sharing events occurred between query plasmids and chromosomes (Table 2; note that the parameters used for the search were conservative and would identify only the most obvious events). Each event was observed in one to nine isolates and involved up to 117 genes. Notably, three sharing events included toxin–antitoxin systems, two included metal resistance genes and one included pathogenicity genes. More specifically, event #1 corresponded to the transposon of a copper resistance plasmid carried on the chromosome of several *Stenotrophomonas* isolates. This chromosomal transposon was extensively described previously (Richard et al., 2017) and its detection thus constitutes a proof of concept for our method. A second event corresponded to the known chromosomal integration of the TALE virulence gene *pthAw2* on the chromosome of *Xa. citri* pv. *citri* isolates from Texas (event #9, Table 2) (Munoz Bodnar et al., 2017), while those effectors were initially thought to be exclusively plasmid-borne in this taxon (Jalan et al., 2014). Interestingly, we found multiple plasmids and chromosomes sharing identical toxin–antitoxin systems. Whereas a plasmid-encoded toxin–antitoxin system constrains the maintenance of a plasmid by a bacterial lineage, its transfer to the chromosome makes the loss of that plasmid possible (Van Melderen & Saavedra De Bast, 2009). While toxin–antitoxin system functions might extend beyond plasmid maintenance (Munoz Bodnar et al., 2017; Van Melderen, 2010; Van Melderen & Saavedra De Bast, 2009), the characterization of the dynamics of their transfer between replicons provides fuel for studies focusing on the paradox of plasmid existence (Carroll & Wong, 2018). Finally, a mercury resistance locus, originally detected on the pLM159.2 plasmid of an *Xa. vesicatoria* tomato isolate (event #2), was detected within the chromosome of four *Pseudomonas* species. The existence of those transfers suggests that other genetic elements, such as transposable elements which can mediate gene mobility between replicons, might also play an important role in microbial evolution (Hooper et al., 2009). Moreover, regardless of those transfers being plasmid

to chromosome or chromosome to plasmid movements, capturing the inter-replicon exchange of such resistance genes is of importance in the global context of antimicrobial resistance monitoring.

4 | CONCLUDING REMARKS

Gene sharing bipartite networks were used to characterize the distribution of plasmid genes of the bacterial family *Lysobacteraceae* with the whole set of available bacterial genomic sequences. Whereas isolates of the family were densely connected by plasmid gene sharing events, a limited number of genes were shared with other families. Analysis of the network structure showed that plasmid genes are more readily shared by bacterial isolates having similar characteristics such as taxonomy or lifestyle. Two network substructures were mostly defined by the sharing of antimicrobial genes. The first included tomato and pepper plant pathogenic bacteria often controlled by pesticides and the second grouped citrus pathogenic species and commensal species known to share heavy metal resistance determinants. Together, those results further highlight the impact of human-mediated selective pressure operating over bacterial pathogenic populations. Lysobacteraceae communities form a unit whose study as a whole makes sense, particularly considering the possible consequences that the spreading of genes among this pathogenic family can have on plant, animal and human health.

AUTHOR CONTRIBUTION

OP, PL, PR and DR designed the research. PL and DR analyzed the data. PL, OP, PR and DR wrote the paper.

ACKNOWLEDGEMENTS

The European Union (ERDF contract GURDT I2016-1731-0006632), Conseil Régional de La Réunion, ANSES and CIRAD provided financial support. D.R. acknowledges a PhD grant from ANSES and CIRAD. This work was supported by Agropolis Fondation (Labex Agro – Montpellier, E-SPACE project no. 1504-004). Analyses were performed using the SouthGreen HPC server.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The dataset analyzed in this study is available on the NCBI databases. Used accessions are listed in the Table S1 and deposited on Zenodo under the DOI 10.5281/zenodo.6531486.

BENEFIT SHARING STATEMENT

There are no benefits to report.

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REFERENCES

Acman, M., van Dorp, L., Santini, J. M., & Balloux, F. (2020). Large-scale network analysis captures biological features of bacterial plasmids. *Nature Communications*, *11*, 2452.

Alavi, S. M., Sanjari, S., Durand, F., Brin, C., Manceau, C., Poussier, S. J. A., & Microbiology, E. (2008). Assessment of the genetic diversity ofXanthomonas axonopodispv. Phaseoli andXanthomonas fuscanssubsp.Fuscansas a basis to identify putative pathogenicity genes and a type III secretion system of the SPI-1 family by multiple suppression subtractive hybridizations. *Applied and Environmental Microbiology*, *74*, 3295–3301.

Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A.-L. V., Cheng, A. A., Liu, S., Min, S. Y., Miroshnichenko, A., Tran, H. K., Werfalli, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., … McArthur, A. G. (2019). CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Research*, *48*, D517–D525.

Avison, M. B., Higgins, C. S., von Heldreich, C. J., Bennett, P. M., & Walsh, T. R. (2001). Plasmid location and molecular heterogeneity of the L1 and L2 β-lactamase genes of *Stenotrophomonas maltophilia*. *Antimicrobial Agents and Chemotherapy*, *45*, 413–419.

Baltrus, D. A., McCann, H. C., & Guttman, D. S. (2017). Evolution, genomics and epidemiology of *pseudomonas syringae*: Challenges in bacterial molecular plant pathology. *Molecular Plant Pathology*, *18*, 152–168.

Banerjee S, Jenamani M, Pratihar DK. 2017. Properties of a projected network of a bipartite network. In *2017 International Conference on Communication and Signal Processing (ICCSP)*, pp. 143–147. https://doi.org/10.1109/ICCSP.2017.8286734

Bennett, P. M. (2004). Genome plasticity: Insertion sequence elements, transposons and integrons, and DNA rearrangement. *Methods in Molecular Biology*, *266*, 71–113.

Berg, G., & Martinez, J. L. J. (2015). Friends or foes: Can we make a distinction between beneficial and harmful strains of the *Stenotrophomonas maltophilia* complex? *Frontiers in Microbiology*, *6*, 241.

Blondel, V. D., Guillaume, J.-L., Lambiotte, R., & Lefebvre, E. (2008). Fast unfolding of communities in large networks. *Journal of Statistical Mechanics: Theory and Experiment*, *2008*, P10008.

Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A., & Bonas, U. (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science*, *326*, 1509–1512.

Carroll, A. C., & Wong, A. (2018). Plasmid persistence: Costs, benefits, and the plasmid paradox. *Canadian Journal of Microbiology*, *64*(5), 293–304. https://doi.org/10.1139/cjm-2017-0609

Che, Y., Yang, Y., Xu, X., Břinda, K., Polz, M. F., Hanage, W. P., & Zhang, T. J. (2021). Conjugative plasmids interact with insertion sequences to shape the horizontal transfer of antimicrobial resistance genes. *Proceedings of the National Academy of Sciences of the United States of America*, *118*, e2008731118.

Chen, N. W., Ruh, M., Darrasse, A., Foucher, J., Briand, M., Costa, J., Studholme, D. J., & MJMPP, J. (2021). Common bacterial blight of bean: A model of seed transmission and pathological convergence. *Molecular Plant Pathology*, *22*, 1464–1480.

Chen, N. W. G., Serres-Giardi, L., Ruh, M., Briand, M., Bonneau, S., Darrasse, A., Barbe, V., Gagnevin, L., Koebnik, R., & Jacques, M. A. (2018). Horizontal gene transfer plays a major role in the pathological convergence of *Xanthomonas* lineages on common bean. *BMC Genomics*, *19*, 606.

Cordero, O. X., & Polz, M. F. (2014). Explaining microbial genomic diversity in light of evolutionary ecology. *Nature Reviews Microbiology*, *12*, 263–273.

Corel, E., Lopez, P., Meheust, R., & Bapteste, E. (2016). Network-thinking: Graphs to analyze microbial complexity and evolution. *Trends in Microbiology*, *24*, 224–237.

Crossman, L. C., Gould, V. C., Dow, J. M., Vernikos, G. S., Okazaki, A., Sebaihia, M., Saunders, D., Arrowsmith, C., Carver, T., Peters, N., Adlem, E., Kerhornou, A., Lord, A., Murphy, L., Seeger, K., Squares, R., Rutter, S., Quail, M. A., Rajandream, M. A., … Avison, M. B. (2008). The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. *Genome Biology*, *9*, R74.

Csardi, G., & Nepusz, T. (2006). The igraph software package for complex network research. *Inter Journal Complex Systems*, *1695*, 1–9.

Curley, C. M., Brodie, E., Lechner, M., & Purcell, A. J. (2007). Exploration for facultative endosymbionts of glassy-winged sharpshooter (*Hemiptera Cicadellidae*). *Annals of the Entomological Society of America*, *100*, 345–349.

Dagan, T., Artzy-Randrup, Y., & Martin, W. (2008). Modular networks and cumulative impact of lateral transfer in prokaryote genome evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 10039–10044.

Davies, J. E. (1997). Origins, acquisition and dissemination of antibiotic resistance determinants. *Ciba Foundation Symposium*, *207*, 15–27 discussion 27-35.

De la Cruz, F., & Davies, J. (2000). Horizontal gene transfer and the origin of species: Lessons from bacteria. *Trends in Microbiology*, *8*, 128–133.

Deyett, E., & Rolshausen, P. E. (2019). Temporal dynamics of the sap microbiome of grapevine under high Pierce's disease pressure. *Frontiers in Plant Science*, *10*, 1246.

Dorer, M. S., Fero, J., & Salama, N. R. (2010). DNA damage triggers genetic exchange in *helicobacter pylori*. *PLoS Pathogens*, *6*, e1001026.

Fargier, E., & Manceau, C. (2007). Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris* pv. *Campestris*. *Plant Pathology*, *56*, 805–818.

Garita-Cambronero, J., Palacio-Bielsa, A., López, M. M., & Cubero, J. (2016). Comparative genomic and phenotypic characterization of pathogenic and non-pathogenic strains of *Xanthomonas arboricola* reveals insights into the infection process of bacterial spot disease of stone fruits. *PLoS One*, *11*, e0161977.

Gochez, A. M., Huguet-Tapia, J. C., Minsavage, G. V., Shantaraj, D., Jalan, N., Strauss, A., Lahaye, T., Wang, N., Canteros, B. I., Jones, J. B., et al. (2018). Pacbio sequencing of copper-tolerant *Xanthomonas citri* reveals presence of a chimeric plasmid structure and provides insights into reassortment and shuffling of transcription activator-like effectors among *X. citri* strains. *BMC Genomics*, *19*, 16.

Gogarten, J. P., & Townsend, J. P. (2005). Horizontal gene transfer, genome innovation and evolution. *Nature Reviews Microbiology*, *3*, 679–687.

Gordon, J. L., Lefeuvre, P., Escalon, A., Barbe, V., Cruveiller, S., Gagnevin, L., & Pruvost, O. (2015). Comparative genomics of 43 strains of *Xanthomonas citri* pv. *Citri* reveals the evolutionary events giving rise to pathotypes with different host ranges. *BMC Genomics*, *16*, 1098.

Gullberg, E., Albrecht, L. M., Karlsson, C., Sandegren, L., & Andersson, D. I. (2014). Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. *mBio*, *5*, e01918-14.

Haegeman, B., & Weitz, J. S. (2012). A neutral theory of genome evolution and the frequency distribution of genes. *BMC Genomics*, *13*, 196.

Hail, D., Lauzière, I., Dowd, S. E., & Bextine, B. J. E. E. (2011). Culture independent survey of the microbiota of the glassy-winged sharpshooter (Homalodisca vitripennis) using 454 pyrosequencing. *Environmental Entomology*, *40*, 23–29.

Halary, S., Leigh, J. W., Cheaib, B., Lopez, P., & Bapteste, E. (2010). Network analyses structure genetic diversity in independent genetic worlds. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 127–132.

Hao, W., & Golding, G. B. (2006). The fate of laterally transferred genes: Life in the fast lane to adaptation or death. *Genome Research*, *16*, 636–643.

Harrison, E., & Brockhurst, M. A. (2012). Plasmid-mediated horizontal gene transfer is a coevolutionary process. *Trends in Microbiology*, *20*, 262–267.

Hobman, J. L., & Crossman, L. C. (2015). Bacterial antimicrobial metal ion resistance. *Journal of Medical Microbiology*, *64*, 471–497.

Hooper, S. D., Mavromatis, K., & Kyrpides, N. C. (2009). Microbial co-habitation and lateral gene transfer: What transposases can tell us. *Genome Biology*, *10*, R45.

Huang, W., Reyes-Caldas, P., Mann, M., Seifbarghi, S., Kahn, A., Almeida, R. P., Béven, L., Heck, M., Hogenhout, S., & Coaker, G. J. (2020). Bacterial vector-borne plant diseases: Unanswered questions and future directions. *Molecular Plant*, *13*, 1379–1393.

Hyatt, D., Chen, G. L., Locascio, P. F., Land, M. L., Larimer, F. W., & Hauser, L. J. (2010). Prodigal: Prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics*, *11*, 119.

Hynes, A. P., Shakya, M., Mercer, R. G., Grüll, M. P., Bown, L., Davidson, F., Steffen, E., Matchem, H., Peach, M. E., & Berger, T. (2016). Functional and evolutionary characterization of a gene transfer agent's multilocus "genome". *Molecular Biology and Evolution*, *33*, 2530–2543.

Hyun, J.-W., Kim, H.-J., Yi, P.-H., Hwang, R.-Y., & Park, E.-W. (2012). Mode of action of streptomycin resistance in the citrus canker pathogen (*Xanthomonas smithii* subsp. *citri*) in Jeju Island. *The Plant Pathology Journal*, *28*, 207–211.

Jalan, N., Yan, Q., Kogenaru, S., Guo, Y., Jones, J. B., Graham, J. H., & Wang, N. (2014). Genomics of *Xanthomonas citri* and related species. In D. C. Gross, A. Lichens-Park, & C. Kole (Eds.), *Genomics of plant-associated bacteria* (pp. 151–176). Springer.

Jibrin, M. O., Potnis, N., Timilsina, S., Minsavage, G. V., Vallad, G. E., Roberts, P. D., Jones, J. B., & Goss, E. M. (2018). Genomic inference of recombination-mediated evolution in *Xanthomonas euvesicatoria* and *X. perforans*. *Applied and Environmental Microbiology*, *84*(13), e00136-18.

Kanamori, H., Yano, H., Tanouchi, A., Kakuta, R., Endo, S., Ichimura, S., Ogawa, M., Shimojima, M., Inomata, S., & Ozawa, D. (2015). Prevalence of Smqnr and plasmid-mediated quinolone resistance determinants in clinical isolates of *Stenotrophomonas maltophilia* from Japan: Novel variants of Smqnr. *New Microbes and New Infections*, *7*, 8–14.

Lang, A. S., Westbye, A. B., & Beatty, J. T. (2017). The distribution, evolution, and roles of gene transfer agents in prokaryotic genetic exchange. *Annual Review of Virology*, *4*, 87–104.

Leyns, F., De Cleene, M., Swings, J. G., & De Ley, J. (1984). The host range of the genus *Xanthomonas*. *The Botanical Review*, *50*, 308–356.

Liebert, C. A., Hall, R. M., & Summers, A. O. (1999). Transposon Tn21, flagship of the floating genome. *Microbiology and Molecular Biology Reviews*, *63*, 507–522.

McInerney, J. O. (2013). More than tree dimensions: Inter-lineage evolution's ecological importance. *Trends in Ecology & Evolution*, *28*, 624–625.

Minsavage, G. V., Canteros, B. I., & Stall, R. E. (1990). Plasmid-mediated resistance to streptomycin in *Xanthomonas campestris* pv. *Vesicatoria*. *Phytopathology*, *80*, 719–723.

Munoz Bodnar, A., Santillana, G., Mavrodieva, V., Liu, Z., Nakhla, M., & Gabriel, D. W. (2017). Complete genome sequences of three *Xanthomonas citri* strains from Texas. *Genome Announcements*, *5*, e00609-17.

Nakato, V., Mahuku, G., & Coutinho, T. (2018). *Xanthomonas campestris* pv. *Musacearum*: A major constraint to banana, plantain and

enset production in central and East Africa over the past decade. *Molecular Plant Pathology*, *19*, 525–536.

Naushad, S., Adeolu, M., Wong, S., Sohail, M., Schellhorn, H. E., & Gupta, R. S. (2015). 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*, *107*, 467–485.

Newman, M. E. J. (2003). Mixing patterns in networks. *Physical Review E*, *67*, 026126.

Niu, X.-N., Wei, Z.-Q., Zou, H.-F., Xie, G.-G., Wu, F., Li, K.-J., Jiang, W., Tang, J.-L., & Y-QJ, H. (2015). Complete sequence and detailed analysis of the first indigenous plasmid from Xanthomonas oryzae pv. Oryzicola. *BMC Microbiology*, *15*, 1–15.

Ochman, H., Lawrence, J. G., & Groisman, E. A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature*, *405*, 299–304.

O'Donovan, C., Martin, M. J., Gattiker, A., Gasteiger, E., Bairoch, A., & Apweiler, R. (2002). High-quality protein knowledge resource: SWISS-PROT and TrEMBL. *Briefings in Bioinformatics*, *3*, 275–284.

Okinaka, R. T., Cloud, K., Hampton, O., Hoffmaster, A. R., Hill, K. K., Keim, P., Koehler, T. M., Lamke, G., Kumano, S., Mahillon, J., Manter, D., Martinez, Y., Ricke, D., Svensson, ǂ. R., & Jackson, P. J. (1999). Sequence and organization of pXO1, the large *bacillus anthracis* plasmid harbouring the anthrax toxin genes. *Journal of Bacteriology*, *181*, 6509–6515.

Osdaghi, E., Jones, J. B., Sharma, A., Goss, E. M., Abrahamian, P., Newberry, E. A., Potnis, N., Carvalho, R., Choudhary, M., Paret, M. L., Timilsina, S., & Vallad, G. E. (2021). A centenary for bacterial spot of tomato and pepper. *Molecular Plant Pathology*, *22*, 1500–1519.

Polz, M. F., Alm, E. J., & Hanage, W. P. (2013). Horizontal gene transfer and the evolution of bacterial and archaeal population structure. *Trends in Genetics*, *29*, 170–175.

Popa, O., & Dagan, T. (2011). Trends and barriers to lateral gene transfer in prokaryotes. *Current Opinion in Microbiology*, *14*, 615–623.

Popa, O., Hazkani-Covo, E., Landan, G., Martin, W., & Dagan, T. (2011). Directed networks reveal genomic barriers and DNA repair bypasses to lateral gene transfer among prokaryotes. *Genome Research*, *21*, 599–609.

Pradier, L., Tissot, T., Fiston-Lavier, A.-S., & Bedhomme, S. (2021). PlasForest: A homology-based random forest classifier for plasmid detection in genomic datasets. *BMC Bioinformatics*, *22*, 349.

Pruvost, O., Magne, M., Boyer, K., Leduc, A., Tourterel, C., Drevet, C., Ravigne, V., Gagnevin, L., Guerin, F., Chiroleu, F., Koebnik, R., Verdier, V., & Vernière, C. (2014). A MLVA genotyping scheme for global surveillance of the citrus pathogen *Xanthomonas citri* pv. *Citri* suggests a worldwide geographical expansion of a single genetic lineage. *PLoS One*, *9*, e98129.

Punina, N., Ignatov, A., Pekhtereva, E. S., Kornev, K., Matveeva, E., Polityko, V., Budenkov, N., & Schaad, N. (2007). Occurrence of *Xanthomonas campestris* pv. *Raphani* on tomato plants in The Russian Federation. *II International Symposium on Tomato Diseases*, *808*, 287–290.

Richard, D., Ravigne, V., Rieux, A., Facon, B., Boyer, C., Boyer, K., Grygiel, P., Javegny, S., Terville, M., Canteros, B. I., Robène, I., Vernière, C., Chabirand, A., Pruvost, O., & Lefeuvre, P. (2017). Adaptation of genetically monomorphic bacteria: Evolution of copper resistance through multiple horizontal gene transfers of complex and versatile mobile genetic elements. *Molecular Ecology*, *26*, 2131–2149.

Rodríguez-Beltrán, J., DelaFuente, J., León-Sampedro, R., MacLean, R. C., & San Millán, Á. (2021). Beyond horizontal gene transfer: The role of plasmids in bacterial evolution. *Nature Reviews Microbiology*, *19*, 347–359.

Rogers, E. E., & Stenger, D. C. (2012). A conjugative 38 kB plasmid is present in multiple subspecies of *Xylella fastidiosa*. *PLoS One*, *7*, e52131.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahe, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584.

Roux, B., Bolot, S., Guy, E., Denancé, N., Lautier, M., Jardinaud, M.-F., Fischer-Le Saux, M., Portier, P., Jacques, M.-A., & Gagnevin, L. (2015). Genomics and transcriptomics of *Xanthomonas campestris* species challenge the concept of core type III effectome. *BMC Genomics*, *16*, 975.

Ryan, R. P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M. B., Berg, G., Van Der Lelie, D., & Dow, J. M. J. N. R. M. (2009). The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nature Reviews Microbiology*, *7*, 514–525.

Seitz, P., & Blokesch, M. (2013). Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental gram-negative bacteria. *FEMS Microbiology Reviews*, *37*, 336–363.

Shapiro, B. J., Friedman, J., Cordero, O. X., Preheim, S. P., Timberlake, S. C., Szabó, G., Polz, M. F., & Alm, E. J. (2012). Population genomics of early events in the ecological differentiation of bacteria. *Science*, *336*, 48–51.

Sicard, A., Zeilinger, A. R., Vanhove, M., Schartel, T. E., Beal, D. J., Daugherty, M. P., & Almeida, R. P. P. (2018). *Xylella fastidiosa*: Insights into an emerging plant pathogen. *Annual Review of Phytopathology*, *56*, 181–202.

Skippington, E., & Ragan, M. A. (2012). Phylogeny rather than ecology or lifestyle biases the construction of *Escherichia coli–shigella* genetic exchange communities. *Open Biology*, *2*, 120112.

Smillie, C., Garcillan-Barcia, M. P., Francia, M. V., Rocha, E. P. C., & de la Cruz, F. (2010). Mobility of plasmids. *Microbiology and Molecular Biology Reviews*, *74*, 434.

Staehlin, B. M., Gibbons, J. G., Rokas, A., O'Halloran, T. V., & Slot, J. C. (2016). Evolution of a heavy metal homeostasis/resistance Island reflects increasing copper stress in *enterobacteria*. *Genome Biology and Evolution*, *8*, 811–826.

Steinegger, M., & Soding, J. (2017). MMseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. *Nature Biotechnology*, *35*, 1026–1028.

Tamminen, M., Virta, M., Fani, R., & Fondi, M. (2011). Large-scale analysis of plasmid relationships through gene-sharing networks. *Molecular Biology and Evolution*, *29*, 1225–1240.

Tenaillon, O., Skurnik, D., Picard, B., & Denamur, E. (2010). The population genetics of commensal *Escherichia coli*. *Nature Reviews Microbiology*, *8*, 207–217.

Thomas, C. M., & Nielsen, K. M. (2005). Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nature Reviews Microbiology*, *3*, 711–721.

Touchon, M., de Sousa, J. A. M., & Rocha, E. P. (2017). Embracing the enemy: The diversification of microbial gene repertoires by phage-mediated horizontal gene transfer. *Current Opinion in Microbiology*, *38*, 66–73.

Urban, M., Cuzick, A., Seager, J., Wood, V., Rutherford, K., Venkatesh, S. Y., De Silva, N., Martinez, M. C., Pedro, H., Yates, A. D., Hassani-Pak, K., & Hammond-Kosack, K. E. (2019). PHI-base: The pathogen–host interactions database. *Nucleic Acids Research*, *48*, D613–D620.

Van Melderen, L. (2010). Toxin–antitoxin systems: Why so many, what for? *Current Opinion in Microbiology*, *13*, 781–785.

Van Melderen, L., & Saavedra De Bast, M. (2009). Bacterial toxin-antitoxin systems: More than selfish entities? *PLoS Genetics*, *5*, e1000437.

Wiedenbeck, J., & Cohan, F. M. (2011). Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. *FEMS Microbiology Reviews*, *35*, 957–976.

Yang, Y. (1994). Host-specific symptoms and increassed release of *Xanthomonas citri* and *X. campestris* pv. *Malvacearum* from leaves are determined by the 102-bp tandem repeats of pthA and avrb6, respectively. *Molecular Plant-Microbe Interactions*, *7*, 345–355.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Richard, D., Roumagnac, P., Pruvost, O., & Lefeuvre, P. (2022). A network approach to decipher the dynamics of Lysobacteraceae plasmid gene sharing. *Molecular Ecology*, *00*, 1–14. https://doi.org/10.1111/mec.16536