

# Genome Sequence-based Insights into the Biology of the Sugarcane Pathogen *Leifsonia xyli* subsp. *xyli*

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## Abstract

*Leifsonia xyli* subsp. *xyli* (*Lxx*) causes ratoon stunting disease (RSD), a major worldwide disease of sugarcane. Formerly classified as *Clavibacter xyli* subsp. *xyli*, *Lxx* is a fastidious member of the GC-rich Actinomycetales, a taxonomic order that comprises two other genera of plant pathogens of great agricultural impact. In this review we present some interesting features of the genome of *Lxx* with emphasis on pathogenicity. Most striking is the observation that *Lxx* has a relatively large number of pseudogenes suggestive of an ongoing process of genome decay. It has been proposed that *Lxx* was once a free-living bacterium that is now restricted to the xylem as a consequence or cause of the accumulation of pseudogenes. This point stems from the observation that although *Lxx* has only been detected inhabiting the xylem of sugar cane, it carries several genes typical of free-living organism. In this review we also discuss the relevance of lateral gene transfer in the acquisition of a few genes associated with pathogenicity and the contribution of mobile genetic elements.

## Introduction

*Leifsonia xyli* subsp. *xyli* (*Lxx*) is a small, fastidious, Gram-positive, coryneform bacterium that causes the ratoon stunting disease (RSD) of sugarcane. *Lxx* belongs to the phylum Actinobacteria, which is the largest taxonomic unit within the domain of Bacteria. Members of this group inhabit a variety of environments and have different lifestyles (Ventura *et al.*, 2007). Species of the genus *Mycobacterium*, for instance, are the

most studied due to their importance as human and animal pathogens (Marri *et al.*, 2006) and various virulence factors are well known. However, the same is not true for a group of species of this phylum that are plant pathogens, such as *Lxx*, and some species of *Clavibacter* (Gartemann *et al.*, 2003), *Curtobacterium* (Davis and Vidaver, 2001), and *Streptomyces* (Loria *et al.*, 2006). Despite the significant yield losses related to their attack of crops such as beans, beets, potato, tomato, soybean and sugarcane, little is known about their biology and the mechanisms of interaction with their plant hosts.

Biological studies with *Lxx* have mostly been impaired by its 'misbehaviour' in the laboratory. *Lxx* is renowned for its fastidious growth habit and nutritional requirements, taking 4 weeks for single colonies to be visible on solid medium and 2 weeks for reasonable growth in liquid medium. There is no defined minimal medium and although transposon mutagenesis has been accomplished, it was with great difficulty (Brumbley *et al.*, 2002). As a result of the effort to gather genomic information on different plant related Actinobacteria, complete genome sequencing projects were undertaken or are in progress for *Lxx* (Monteiro-Vitorello *et al.*, 2004), *Clavibacter michiganensis* subsp. *michiganensis* ([https://www.genetik.uni-bielefeld.de/GenoMik/partner/bi\\_eichen.html](https://www.genetik.uni-bielefeld.de/GenoMik/partner/bi_eichen.html)); *Clavibacter michiganensis* subsp. *sepedonicus* ([http://www.sanger.ac.uk/Projects/C\\_michiganensis/](http://www.sanger.ac.uk/Projects/C_michiganensis/)), and *Streptomyces scabies* ([http://www.sanger.ac.uk/Projects/S\\_scabies/](http://www.sanger.ac.uk/Projects/S_scabies/)). *Lxx* is the only finished and annotated plant associated Actinobacterial genome at the

time of writing. It is interesting to note that out of the more than two hundred Actinobacterial genomes sequenced or in the process of being sequenced (<http://www.genomesonline.org/>), only four are plant pathogens and another four are plant-associated bacteria. We expect that the availability of the genome sequence of *Lxx* will be of help to identify virulence factors as well as key genes that allow *Lxx* to live in the xylem of sugarcane, which is a particularly inhospitable environment compared to other existent niches within a plant host. This chapter reviews some of the inferences and hypotheses on the biology of this organism made possible by 'reading' its genome sequence.

### **A brief history of *Leifsonia* as a pathogen**

Formerly classified as *Clavibacter xyli* subsp. *xyli* (Davis *et al.*, 1984), *Lxx* was removed from the genus *Clavibacter* to create the genus *Leifsonia*, together with *L. poae*, found in *Poa annua* root galls, and *L. aquatica*, a free-living bacterium (Evtushenko *et al.* 2000). So far, *Lxx* has been reported as only infecting sugarcane. The disease it provokes (RSD) is found in most sugarcane growing areas of the world and can cause yield losses of up to 30% in susceptible varieties (Gillaspie and Teakle, 1989). Since the bacterium is present in the liquids of infected plants, it can be mechanically disseminated after contamination of the cane knives used in harvesting. Thus, the incidence of infected plants increases during successive ratoon crops. For this reason and for quite some time, cumulative losses due to RSD have probably been greater than the losses caused by any other sugarcane disease (Gillaspie and Teakle, 1989). Curiously, despite being a worldwide presence in commercial fields, *Lxx* has never been found affecting wild clones of *Saccharum officinarum* in its centre of diversity (Magarey *et al.*, 2002), Papua New Guinea, which suggests it has recently evolved to infect its host. The disease was primarily observed in the 1940s, after the production of the first hybrids, based on breeding of *S. officinarum* and *S. spontaneum*. Therefore, RSD may be favoured by the establishment of modern worldwide commercial crops of sugarcane (Brumbley *et al.*, 2006). *Lxx* is believed to have evolved from a single patho-

genic clone, since no genetic variation was found among different isolates of distinct countries and cultivars (Young *et al.*, 2006).

*Lxx* may be regarded as a stealth pathogen since the symptoms it causes are subtle compared to the ones caused by necrogenic bacteria and may be confounded with symptoms caused by other biotic and abiotic stresses. Moreover, symptoms are highly dependent on the genetic background of the varieties and on environmental conditions. Because of this, the spread of RSD through planting material has beset most sugarcane growing areas in the world. Infected plants show reduced cane diameter and shortening of the internodes, i.e., stunting. Discolouration of vascular bundles of mature stalks may be seen in the form of discrete rosy dots or streaks just below the internodes where the bundles branch into the leaf sheath (Gillaspie and Teakle, 1989), but this is of little diagnostic value as infection by other pathogens may cause the same symptom. Internally, *Lxx* colonizes the lumen and the pits of the xylem cells, but not the phloem or parenchyma (Weaver *et al.*, 1977). Thus, *Lxx* does not access the valuable carbon source accumulated by sugarcane but rather lives in the relative nutritionally poor environment of the xylem. No general tissue disorganization or necrosis results from the colonization of the xylem vessels by *Lxx*, although the xylem conduits may be blocked with a mucilaginous substance probably produced by the host. This blockage is reported to reduce sap flow up to 34% (Teakle and Appleton, 1978; Kao and Damann, 1980) which in its turn may result in wilting but only during extensive drought (James, 1996).

Diagnosis is based on the detection of the bacteria by a number of different techniques, including phase contrast microscopy (Steindl, 1976), serology (Gillaspie, 1978) and PCR (Pan *et al.*, 1998). The successful growth of axenic cultures of *Lxx* on specific media (Davis *et al.*, 1980) has made it possible to produce specific antibodies useful for diagnosis.

Since sugarcane is vegetatively propagated and due to the nature of the transmission of *Lxx*, control measures rely primarily on using healthy stalks as planting material. These are raised in special disease-free nurseries in which all cutting instruments are disinfected either by heating

in a flame or by dipping in a chemical solution. Moreover, before planting in the nurseries, these 'seed' stalks are dipped in hot water so as to eliminate the bacteria.

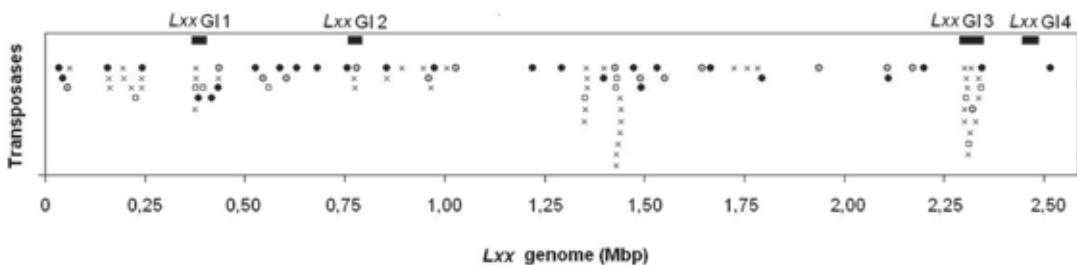
### General features of the genome of *Lxx*

The genome of *Lxx* is 2,584,158 bp in length and is characterized by a high content of G and C bases (67.7%). The total number of predicted genes is 2,351, which were divided into two classes: 2044 intact protein-coding genes and 307 pseudogenes (Monteiro-Vitorello *et al.*, 2004). Pseudogenes were considered as those disrupted by one or more authentic frameshifts and/or a point mutation that introduced a stop codon in frame, or genes that are partially represented based on BLAST results. There is only one copy of the ribosomal genes, 45 tRNAs representing all amino acids, and one tmRNA. A striking feature is the presence of a large number of insertion sequences (IS elements) some of which are within genes, probably impairing their function. Fifty-one genes are predicted to encode transposases located within copies of six IS elements (IS*Lxx*1–6) (Monteiro-Vitorello *et al.*, 2004; Zerillo *et al.*, submitted) distributed along the chromosome (Fig. 6.1). The IS elements belong to the major families IS110, IS21, IS481, IS30 and IS5, as described by Mahillon and Chandler (1998). Two IS elements had a clear recent expansion since all copies are nearly identical (IS*Lxx*4 – 26 copies; and IS*Lxx*5 – 15 copies). Nine elements were found inserted within genes and 14 were in close vicinity of housekeeping pseudogenes. Another 37 transposases were

not classified into one of the six IS elements described and were primarily associated with genomic islands (see discussion below). Sixteen transposases were found clustered in one putative genomic island (LxxGI3), 11 of which are uncharacterized elements of families IS256, IS3, IS481 and IS110 – five of these belong to insertion families IS*Lxx*1, 2, 4, 5 and 6.

### The theory of a recent niche conversion and genome decay

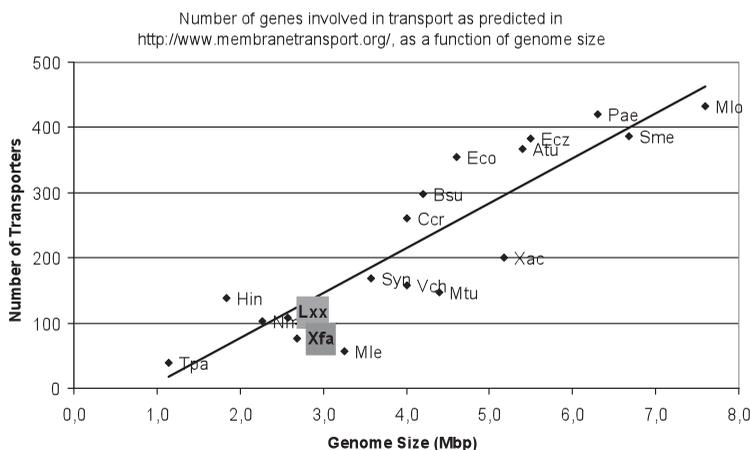
It has been proposed that the accumulation of pseudogenes adversely affects the ability of some obligate parasites and symbionts to colonize diverse niches (Cole *et al.*, 2001; Babu, 2003). As mentioned before, although *Lxx* is cultivable but fastidious, its niche is very restricted since it colonizes only the xylem vessels of sugarcane. A central hypothesis that our research group has proposed after analyzing the *Lxx* genome sequence is that *Lxx* was once a free-living bacterium that is now confined to the xylem and the consequent confinement may be the cause or the result (or probably both) of the accumulation of pseudogenes. This reduction process due to gene loss and its biological consequences, known as genome decay, would be similar to that proposed for the related species *Mycobacterium leprae* (Cole *et al.*, 2001) and for several other obligate pathogens and endosymbionts (Thomson *et al.*, 2003). According to our hypothesis, the loss of functionality of important genes would have restricted the ability of *Lxx* to live as a free-living bacterium as other species of *Leifsonia* do. This initial niche restriction by its turn would then have resulted in a neutral selection pressure against mutations on



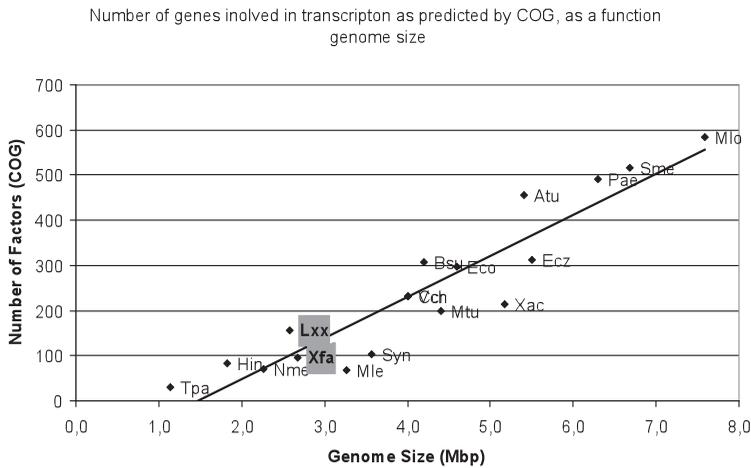
**Figure 6.1** Localization of transposase-encoding genes represented on the complete nucleotide sequence of the *Lxx* genome. Genes separated by less than 20 kbp are shown on different levels for ease of visualization. The most abundant IS elements (IS*Lxx*4 and IS*Lxx*5) are represented as black and grey circles, respectively. Other characterized IS elements are represented as white squares; and transposases of uncharacterized IS elements are indicated by 'X's. The genomic islands (LxxGI1–4) are shown as thick black lines.

genes that were no longer necessary for the free-living lifestyle, thus resulting in the accumulation of pseudogenes (Lawrence *et al.*, 2001). Clues of this ancient free-living lifestyle of *Lxx*, can still be found in the genome of *Lxx* as genes that code for proteins that would apparently be necessary mostly for a free-living bacteria such as a light inducible photolyase, a (non-functional) flagellar operon, a threolose synthase, and genes that code for mechanosensitive channels and transporters, including ones for glycine and betaine, which are typically involved in tolerance to osmotic stress. In addition, *Lxx* has a strikingly large repertoire of ABC transporters compared with *Xylella fastidiosa* (42 as opposed to 26) (Ren *et al.*, 2007) (Fig. 6.2), another xylem-limited plant pathogen, and a complete phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS), which is absent in *X. fastidiosa*. Together, these systems would enable *Lxx* to uptake a range of carbohydrates. However, the presence of this arsenal of sugar transporters is in sharp contrast to the carbon-poor content of the xylem sap of sugarcane (Dong *et al.*, 1997).

Perhaps the most striking suggestion of an early free-living habit of *Lxx* is the finding that as much as 6.9% of all genes are predicted to be transcriptional regulators based on the presence of regulatory motifs (Monteiro-Vitorello *et al.*, 2004). This high number is typical of free-living bacteria, as organisms that live in complex environments need to respond to diverse stimuli compared to those organisms restricted to a more uniform environment (Stover *et al.*, 2000). For instance, in *Pseudomonas aeruginosa* and in *Streptomyces coelicolor*, which are free-living, this percentage is 12% and 9.8%, respectively, whereas in *X. fastidiosa* (which has a genome size equivalent to that of *Lxx*) it is only 3.7%. This same percentage is found in the obligate parasite *M. leprae*. Counting genes associated with transcription as predicted by COG, the differences mentioned were also detected (Fig. 6.3). Among the 173 total regulators found in *Lxx*, 10 are predicted to be non-functional, which is also consistent with our theory of a genome decay process and niche restriction. Analyzing *Lxx* and *X. fastidiosa*, the over representations of tran-



**Figure 6.2** Number of genes associated with membrane transport as found in TransporterDB (<http://www.membranetransport.org/index.html>) (Ren *et al.*, 2007) as a function of genome size. Among more than five hundred genomes completely sequenced, we chose to represent those organisms with different genome sizes and lifestyle, to emphasize a general tendency of bacterial genomes and number of genes associated with membrane transport and lifestyles. Below are the abbreviations used to represent each of the organisms: Tpa, *Treponema pallidum* Nichols; Hin, *Haemophilus influenzae* KW20; Nme, *Neisseria meningitidis* MC58; Lxx, *Leifsonia xyli* subsp. *xyli* CTCB07; Xfa, *Xylella fastidiosa* 9a5c; Mle, *Mycobacterium leprae* TN; Syn, *Synechocystis* sp. PCC6803; Vch, *Vibrio cholerae* El Tor 16961; Ccr, *Caulobacter crescentus* CB15; Bsu, *Bacillus subtilis* 168; Mtu, *Mycobacterium tuberculosis* H37Rv; Eco, *Escherichia coli* K12-MG1655; Xac, *Xanthomonas axonopodis* pv. *citri* 306; Atu, *Agrobacterium tumefaciens* C58; Ecz, *Escherichia coli* O157:H7 EDL933; Pae, *Pseudomonas aeruginosa* PAO1; Sme, *Sinorhizobium meliloti* 1021; Mlo, *Mesorhizobium loti* MAFF303099.



**Figure 6.3** Number of genes involved in transcription as predicted by COG (cluster of orthologous groups), as a function of genome size. Among the more than five hundred genomes completely sequenced, we chose to represent those organisms with different genome sizes and lifestyle, to emphasize a general tendency of bacterial genomes and number of genes associated with transcription and lifestyles. Below are the abbreviations used to represent each of the organisms: Tpa, *Treponema pallidum* Nichols; Hin, *Haemophilus influenzae* KW20; Nme, *Neisseria meningitidis* MC58; Lxx, *Leifsonia xyli* subsp. *xyli* CTCB07; Xfa, *Xylella fastidiosa* 9a5c; Mle, *Mycobacterium leprae* TN; Syn, *Synechocystis* sp. PCC6803; Vch, *Vibrio cholerae* El Tor 16961; Ccr, *Caulobacter crescentus* CB15; Bsu, *Bacillus subtilis* 168; Mtu, *Mycobacterium tuberculosis* H37Rv; Eco, *Escherichia coli* K12-MG1655; Xac, *Xanthomonas axonopodis* pv. *citri* 306; Atu, *Agrobacterium tumefaciens* C58; Ecz, *Escherichia coli* O157:H7 EDL933; Pae, *Pseudomonas aeruginosa* PAO1; Sme, *Sinorhizobium meliloti* 1021; Mlo, *Mesorhizobium loti* MAFF303099.

scriptural regulators in *Lxx* were mainly found in LuxR, ROK, GntR, LacI and TetR regulator families (Monteiro-Vitorello *et al.*, 2004).

Other truncated proteins which were considered to be non-functional are involved in the synthesis of methionine and cysteine. Although cysteine biosynthesis could occur via an atypical pathway similar to that of *Bifidobacterium longum*, which includes cystathionine  $\gamma$ - and  $\beta$ -synthases and cystathionine  $\gamma$ -lyase (Schell *et al.*, 2002), the more common sulphite/sulphate pathway is affected by the inactivation of cysteine synthase (*cysK*). This explains the need for cysteine in the standard *Lxx* culture medium developed by Davis and collaborators (1980). Also genes *metE* and *metF*, both involved in the folate branch of the methionine biosynthetic pathway, are truncated. In *Streptomyces lividans*, disruption of *metF* led to methionine auxotrophy (Blanco *et al.*, 1998) and this might be the case of *Lxx* since its growth is greatly improved in liquid medium by the addition of this amino acid (Monteiro-Vitorello *et al.*, 2004).

### Lateral transfer and pathogenicity

Analysis of codon bias, GC composition and dinucleotide signatures indicated the existence of anomalous regions in the genome of *Lxx* that is suggestive of lateral transfer. Four regions were defined as genomic islands and named *LxxGI1-LxxGI4* (Table 6.1) (Monteiro-Vitorello *et al.*, 2004). Regions *LxxGI2*, *LxxGI3* and *LxxGI4* contain genes known to be involved in pathogenicity in other organisms. *LxxGI1* and *LxxGI4* are probably of phage origin, due to the presence of phage-related genes. In both cases we defined the site of insertion and the duplicated sequence upon insertion (Fig. 6.4).

Genomic island *LxxGI2* could be the result of a plasmid integration event, since it harbours a relaxase/mobilization (Rlx) protein required for the horizontal transfer of plasmids during bacterial conjugation (Parker *et al.*, 2005). In islands *LxxGI2* and *LxxGI3*, genes were found coding for a pectinase and a cellulase, respectively, enzymes which are present in variable number of copies in bacterial plant pathogens (Van Sluys *et al.*, 2002). It has been speculated that these genes



besides playing a regulatory and signalling role in plants against abiotic stresses, ABA also appears to play such roles during pathogen attack. Audenaert *et al.* (2002) demonstrated that an ABA deficient tomato mutant is more resistant to the fungus *Botrytis cinerea* than the wild-type and that this can be reversed by exogenous application of ABA. Exogenous treatment with this hormone also rendered *Arabidopsis* plants susceptible to *Pseudomonas syringae* pv. *tomato* and this was related to suppression of the accumulation of disease-resistance related compounds (Mohr and Cahill, 2007). The putative carotenoid biosynthetic pathway of *Lxx* is represented by five essential genes that would enable this bacterium to produce  $\beta$ -carotene from geranyl pyrophosphate (GPP) and isopentenyl pyrophosphate (Armstrong, 1997). These genes are closely related to the *crt* operon of *Brevibacterium linens* (Krubasik and Sandmann, 2000) and *Corynebacterium glutamicum* (Krubasik *et al.*, 2001). We are currently testing the functionality of this gene cluster by heterologous expression in non-carotenogenic bacteria, such as *Escherichia coli*.

*LxxGI4* harbours a gene similar to the *pat-1* gene of *Clavibacter michiganensis* subsp. *michiganensis*, a pathogen of tomatoes. In this organism, this plasmid-encoded gene plays a decisive role in causing plant wilting (Dreier *et al.*, 1997). Upon transformation, mildly virulent strains which lack this gene and do not cause wilting, acquire the wilt-inducing phenotype. Also, this phenotype is attenuated if a repetitive sequence positioned downstream of the gene (*pat-1rep*) is deleted. Thus it may be regarded as a major determinant of virulence of this closely related bacterium. In *Lxx*, the *pat-1* homologue (*e*-value  $5e-15$ ) is present in two copies, although only the one present in *LxxGI4* may be functional. This copy has the consensus motif of the trypsin family of serine proteases characteristic of *pat-1*, but lacks the downstream repetitive sequence. The absence of *pat-1rep* in *Lxx* might be the reason why this pathogen causes wilting only in highly susceptible sugarcane varieties under very particular environmental conditions.

Given the primary roles of the genes discussed above in the pathogenicity of various plant pathogens, they would be suitable candidates for

further functional studies aiming to determine the virulence mechanisms of *Lxx*. Also, from this analysis, it is clear that lateral gene transfer has played a central role in shaping the pathogenicity of *Lxx* CTCB07.

In addition to potential virulence genes located in genomic islands, *Lxx* encodes genes implicated in pathogenicity in other bacterial pathogens distributed throughout the genomic sequence. These include haemolysins similar to the *tlyA* and *tlyC* widely present in pathogens as well as a haemolysin III homologue. The type III secretory system is absent, as expected for a Gram-positive bacterium. Also absent is the cytolysin-mediated translocation system, which is predicted to be the counterpart of this system in Gram-positive bacteria (Madden *et al.*, 2001). Noteworthy is the presence of a flagellar operon comprising 17 genes encoding proteins of the basal body (MotA, FlgC, F, FliE, G, O), a sigma-like transcriptional factor (FliA), hook-assembly proteins (FlgD, E, K, L, FliK), flagellar filament proteins (FliC, D, FlhA), and a flagellum-specific ATP-synthase (FliI). However, notably absent or predicted to be non-functional are four genes involved in the assembly of the flagellar filament (FlgK, FliD, FliK) and exportation of flagellin (FlhA). Consistent with these findings, electron microscopy indicates that *Lxx* lacks flagella (Davis *et al.*, 1984). Since flagellar proteins have been shown to play important roles in host-parasite interactions such as adhesion and transfer of virulence proteins to animal host cells (Arora *et al.*, 1998) and induction of rapid cell death in non-host plants (Taguchi *et al.*, 2003), it would be interesting to determine whether intact operons occur in other strains of *Lxx* or related species and to establish the role of such an intact cellular apparatus, if any, in pathogenicity.

Occlusions of xylem vessels are seen in infected sugarcane (Teakle and Appleton, 1978; Kao and Damann, 1980) but these are probably of host origin since electron microscopic studies of xylem extracts indicated that cells of *Lxx* lack an external capsule (Weaver *et al.*, 1977). Moreover, gum producing genes such as the ones found in *Xylella* (Simpson *et al.*, 2000) are missing. The only two clusters of genes found (*xanA* and *xanB* and the *rmlA/B/C/D*) that could be

associated with EPS and/or LPS production remain to be characterized in more detail.

As mentioned before, *Lxx* is a stealth pathogen due to the subtle and non-typical symptoms of the disease it causes. One reason for this could be the small number of putative pathogenicity genes compared to other bacterial plant pathogens. For instance, only 85 intact genes were categorized as pathogenicity genes in *Lxx* (Monteiro-Vitorello *et al.*, 2004), as opposed to 287 in the necrogenic, 'brute-force' and rotting pathogen of cauliflower and cabbages *Xanthomonas campestris* pv *campestris* (da Silva *et al.*, 2002). This number is small even when compared to *X. fastidiosa* 9a5C and Temecula 1 strains (134 and 159 genes, respectively). Although artificial co-inoculations of *Lxx* and *X. albilineans* were never attempted, the two bacteria can co-exist in the same plant as indicated by PCR assays with species-specific primers carried out in our lab (unpublished results).

## Insights to the general biology of *Lxx*

### Energy metabolism

The genome of *Lxx* encodes none of the 14 proteins that compose complex I, which is the first oxidoreductase complex in the respiratory chain. This absence is also evident in *Bacillus*, *Haemophilus* and *Vibrio cholerae* (COG- NCBI). All these organism may transfer the hydrogen from NADH to Ubiquinone using a type II NADH dehydrogenase (EC 1.6.99.3) or a Na<sup>+</sup>-transporting NADH:ubiquinone oxidoreductase. The *Lxx* genome has only one type II NADH dehydrogenase (ORF 17150.1), which is highly similar to a *Streptomyces* enzyme. Other plant pathogens such as *Xanthomonas*, *Ralstonia* and *Xylella* have both the type I and II NADH dehydrogenase. Complex II and III are present and complete. Regarding complex IV, *Lxx* has more options than *Xylella* (Simpson *et al.*, 2000), since it harbours cytochrome C oxidase and cytochrome D Ubiquinol oxidase while *Xylella* has only cytochrome O Ubiquinol oxidase. However, both are less complete than *Xanthomonas* that has the three cytochrome oxidases (da Silva *et al.*, 2002). These differences might be related to more versatility needed by *Xanthomonas* due to

it being an epiphytic organism, while *Xylella* and *Lxx* are limited to the xylem of their plant hosts. It has been pointed out before that the restricted respiratory complex could be an interesting target for drug design (Bhattacharyya *et al.*, 2002) and might also be related with the fastidious behaviour of xylem-limited bacteria.

### Secretion systems

Several genes encoding proteins of the general secretion pathway were identified including *secA* (translocation motor), *secD/F*, *secY*, *secE*, *secG* and *yajC* (translocation channel). Although the SecB chaperone is not represented in the genome, gene products such as Ffh and FtsY may substitute for the SecB function. A type I signal peptidase is present, but a type II peptidase was not identified (Monteiro-Vitorello *et al.*, 2004). For secretion of folded cofactor-containing proteins across the membrane, *Lxx* probably accomplishes it with the twin arginine-motif translocation (TAT) pathway as TAT genes (A–E) are present.

### Adaptation to the host

Albicidin is the major component of a complex of toxins produced by *Xanthomonas albilineans* (Birch and Patil, 1985; 1987ab), another sugarcane bacterial pathogen which inhabits the xylem vessels causing leaf scald disease (Rott *et al.*, 1996). In sugarcane, albicidin inhibits plastid DNA replication, resulting in blocked chloroplast differentiation and the chlorotic streaks that are characteristic of the disease (Birch and Patil, 1985; Birch and Patil, 1987a,b; Birch *et al.*, 1990). *X. albilineans* strains unable to produce albicidin do not induce leaf scald symptoms in sugarcane.

Albicidin is also bactericidal to a large range of Gram positive and Gram negative bacteria pathogenic to humans, animals and plants, and may be important during colonization of sugarcane by inhibiting growth of other xylem invading bacteria. Genes associated with albicidin detoxification are known to be present in *Klebsiella oxytoca* (Walker *et al.*, 1988), *Alcaligenes denitrificans* (Basnayake *et al.*, 1995), *Pantoea dispersa* (syn. *Erwinia herbicola*) (Zhang & Birch, 1997), and also in *X. albilineans*. Four predicted genes similar to those encoding multidrug resist-

ance proteins, and one similar to the *albF* of *X. albilineas* are found in *Lxx*. We speculate that one or more of these genes could be associated with *Lxx* albicidin resistance.

### Concluding remarks

Although genomics does not provide immediate solutions, the science does offer the opportunity to create hypotheses and to design precise experiments. The 'reading' of the *Lxx* genome sequence revealed a wide range of biological issues that can be further addressed. For example, considering the lifestyles of *X. fastidiosa* and *Lxx*: even though both are xylem-limited fastidious plant pathogens and their presence is associated with clogging of xylem vessels, comparative analysis of their genomes revealed that the pool of genes and strategies used by them are probably quite different. *Xylella* seems to be much more specialized to this niche than *Lxx*. Particularly intriguing is the presence of a variable number of transcriptional regulators and transporters. Pathogens with restricted niches appear to have reduced transport capabilities and regulatory potential when compared to bacteria that can be found in a variety of environments. This is in agreement with what it is found for *Xylella*, but not what was found for *Lxx*. The theory of a recent event of niche restriction fits well within this context, along with the large number of pseudogenes still recognized by BLAST searches. A closer analysis of the origin of those pseudogenes may help to understand the evolution of the interaction of *Lxx* and sugarcane. Within the same context, the comparative genomic analysis with *L. xyli* subsp. *cynodontis* (*Lxc*) genome, a bacterium that can be inoculated in the xylem of sugarcane causing none of the RSD disease symptoms would provide valuable new insights on *Lxx* biology. Currently, we are investigating the presence of IS elements associated with specific or rearranged fragments between *Lxx* and *Lxc* genomes (Zerillo *et al.*, unpublished).

As yet, the relationship of *Lxx* and sugarcane is intriguing, as the mechanisms of colonization and symptom-induction are not completely understood. The discrete symptoms of the disease even at high bacterial titres of plant-infection was tentatively explained by the limited number of pathogenicity genes (Monteiro-Vitorello

*et al.*, 2004), which made *Lxx* be regarded as a near-perfect pathogen (Metzler *et al.*, 1997). The evolutionary forces of lateral gene transfer have also played an important role in shaping the *Lxx* genome by restricting the niche as well as bringing in new pathogenicity genes. It is also worthwhile mentioning the presence of *desA* in *Lxx*, suggesting the production of an ABA-like molecule that could help to explain the symptoms of stunting associated with RSD.

*Lxx* used to be part of the *Clavibacter* genus and indeed *Lxx* and *Clavibacter michiganensis* are closer to each other than to any other Actinobacterium with a completely sequenced genome. Preliminary analyses using draft sequences of *C. michiganensis* subsp. *michiganensis* (*Cmm*) and *C. michiganensis* subsp. *sepedonicus* (*Cms*) have shown that none of the *Lxx* genomic islands are present, even though some similarity exists among most of the transposases found in *LxxGI3* and the genome of *Cms*. The cluster of *Lxx* genes associated with flagella assembly is not found in either of the two *Clavibacter* genomes. However, the desaturase *desA* gene is present in the *Clavibacter* genomes and is thus a strong candidate for further experimental analysis.

Some questions about *Lxx* biology have been answered, but many other questions have been raised since finishing the *Lxx* genome sequence. We have presented the information of what is known in terms of crop management of the disease and genomic analysis in this review so other questions can be raised, leading to development of new hypotheses and more studies be devoted to understanding the interaction of *Lxx* and sugarcane.

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