Xylella fastidiosa-Elicited Leaf Scorch Diseases in Taiwan

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ABSTRACT

Xylella fastdiosa is a gram negative, nonflagellate, nutritionally fastidious bacterium that resides only in xylem tissues and requires specific and enriched media for *in vitro* growth. Two *X. fastidiosa*-elicited leaf scorch diseases, pear leaf scorch (PLS) and Pierce's disease (PD), were found in Taiwan in 1991 and 2002. Phylogenetic analyses using 16S rRNA gene and 16S-23S internal transcribed spacer sequence (ITS) revealed that the Taiwan PLS and PD strains may have been independently introduced into and evolved in the host plants. The completion of PLS genome sequences will provide valuable insights into the genome organization and genetic components that diversify PLS bacteria from the other *X. fastidiosa* strains.

Keywords: Pierce's disease, pear leaf scorch disease, shotgun sequencing, genome comparison

The world-wide occurrence of *X. fastidiosa*-induced plant diseases

Xylella fastidiosa is a gram-negative, xylem-limited fastidious bacterium [(13)] that causes numerous scorching, scalding, and stunting diseases worldwide [(4)], and its diverse host range makes the bacterium a serious threat to agricultural activities. Economically important diseases caused by the bacterium include citrus variegated chlorosis (CVC), Pierce's disease of grape (PD), phony peach disease, alfalfa dwarf, periwinkle wilt, and bacterial leaf scorch of almond, coffee, plum, pear, mulberry, elm, oak, sycamore, maple, oleander, pecan, and landscape plants [(4, 8)]. In nature, the bacterium is acquired and transmitted by sharpshooter leafhoppers (*Hemiptera*: Cicadellidae) and spittlebugs (*Hemiptera*: Cercopidae) [(1)] by forming a biofilm of polar-attached cells inside the foreguts of vectors [(6)]. The bacterium can multiply in the

foreguts and be persistently transmitted by adult vectors, and very few live bacteria are required for transmission (for a review, see (1) and references therein).

X. fastidiosa-induced plant diseases are generally found in the region between 15-45 degrees latitude of both north and south of Equator, predominately in North and South Americas. In the US, they occur in the whole southeastern States along the Gulf coast of Mexico, and California. In the southern hemisphere, the diseases occur in Brazil, Argentina, and Paraguay. There is only one report of Pierce's disease of grapevine in Kosovo, the former Yugoslavia, in southern Europe. In 1991, *X. fastidiosa*-induced pear leaf scorch (PLS) disease was found in low altitude areas (below 800 m) in Taiwan where the low-chilling pear cultivar Hengshan (*Pyrus pyrifolia*) was grown, which was the first *X. fastidiosa*-induced plant diseases in Asian Continent [(5)]. Leaf scorch symptoms were observed in early July, 6 months after the sprouting of dormant buds. Accompanying with the scorching symptoms on the leaf margin, early defoliation, declining of tree vigor, dieback and wilting, and significant yield losses in severe cases, pear leaf scorch disease has been a major limiting factor of the pear industry in Taiwan [(9)]. In 2002, another leaf scorch disorder, Pierce's disease (PD) of grapevines, was found in the major grape production areas (Taichung City, Miaoli and Nantou counties) in central Taiwan [(12)]. Scorch symptoms on grapevine leaves were observed at the onset of berry ripening (veraison phase) in late May to early June. Necrotic tissue with yellow or burgundy margins was developed at the edge of the symptomatic leaves and became coalesced in later stage, followed by the systematic development of scorch symptoms in upper and lower leaves. Severely affected leaves became fully necrotic and dropped early, showing matchstick-like petioles attached to the cane. Affected twigs and branches of the grapevines declined and dieback in 1 to 5 years post infection.

The relatedness of Taiwan PD and PLS bacteria with the other *X. fastidiosa* strains

To characterize the genetic relatedness of the *X. fastidiosa* strains isolated in Taiwan, PCR-based DNA amplification and phylogenetic analyses were applied. DNA fingerprinting patterns amplified by arbitrary primers show that PLS strains are genetically distinct from *X. fastidiosa* strains isolated from other host plants [(10)]. Phylogenetic analyses using 16S rRNA gene and 16S-23S internal transcribed spacer sequence (ITS) reveal that the Taiwan PD strains are grouped together with the other

PD strains collected from North and South Americas that belonged to *X. fastidiosa* subsp. *fastidiosa* [8], whereas the PLS strains are distantly related to the PD strains and the other *X. fastidiosa* strains collected from different plant species [11, 12], suggesting the Taiwan *X. fastidiosa* bacteria that infects grapevines and Asian pear might have different origins.

Genome-wide comparison of *X. fastidiosa*

Microarray-based comparisons using strain 9a5c (CVC strain) as a reference genome to be hybridized with 11 *X. fastidiosa* strains reveal that these bacteria display a large set of flexible genes, with several horizontally transferred elements contributing up to 18% of the total genome [7]. Whole genome sequences of *X. fastidiosa* strains, including 5 complete genomic sequences of 9a5c (Citrus variegated chlorosis), Temecula 1 and GB514 (Pierce's disease of grapevine), M12 and M23 (almond leaf scorch), and 2 draft sequences of Dixon (almond leaf scorch disease) and Ann1 (oleander leaf scorch disease), are available (http://www. ncbi.nlm.nih.gov/genome/genomes/173). Comparative analyses of these genome sequences show that 76.2% of the genome sequences are conserved among *X. fastidiosa* strains and identified significant variations among elements coding for additional functions that are not essential for bacterial growth [3]. The overall genomic diversity observed among these *X. fastidiosa* strains provides evidence that different *X. fastidiosa* strains might carry unique genetic factors for adaptability and host specificity.

Analyses of the draft sequences of PLS bacteria

Genomic DNAs of *X. fastidiosa* strains PLS235 and PLS244 were extracted from pure culture in PD2 medium. The random shotgun method was used for genome sequencing, and sequences were de-novo assembled with MIRA assembler and annotated in the web-based RAST server [2]. Nucleotide comparison showed that PLSB whole-genome sequences share approximate 78% similarity with the other *X. fastidiosa* bacteria, suggesting that PLS strains might be a new subspecies of *X. fastidiosa*. Assembly and annotation of PLS235 draft sequences revealed an estimate genome size of 2.99 Mb putatively coding for 3,196 genes. Among the predicted genes, 1,714 genes involved in metabolism, protein synthesis, cell cycle and other house-keeping activities are conserved between PLS235 and *X. fastidiosa* strain 9a5c. INDELs and strain specific genes identified in PLS235 genome are the main source of

variations to differentiate the genetic compositions of *X. fastidiosa* strains, which is similar to the conclusion derived from the genome comparison of *X. fastidiosa* strains Temecula 1, Ann1, Dixon, and 9a5c [3]. Sequence analysis of the PLS235 genomic contigs also identified the association of repeat sequences with hypothetical and phage related functions, and many of them are unique to the PLS genome, suggesting horizontally transferred genes may drive the evolution of *X. fastidiosa* genomes toward metabolically compromised and host-specific strains.

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