Horizontal transfer of potential mobile units in phytoplasmas

Chuan Ku,1 Wen-Sui Lo,1,2,3 and Chih-Horng Kuo1,2,4,*
1Institute of Plant and Microbial Biology; Academia Sinica; Taipei, Taiwan; 2Molecular and Biological Agricultural Sciences Program; Taiwan International Graduate Program; National Chung Hsing University and Academia Sinica; Taipei, Taiwan; 3Graduate Institute of Biotechnology; National Chung Hsing University; Taichung, Taiwan; 4Biotechnology Center; National Chung Hsing University; Taichung, Taiwan

Phytoplasmas are uncultivated phytopathogenic bacteria that cause diseases in a wide range of economically important plants. Through secretion of effector proteins, they are able to manipulate their plant hosts to facilitate their multiplication and dispersal by insect vectors. The genome sequences of several phytoplasmas have been characterized to date and a group of putative composite transposons called potential mobile units (PMUs) are found in these highly reduced genomes. Recently, our team reported the genome sequence and comparative analysis of a peanut witches’ broom (PnWB) phytoplasma, the first representative of the phytoplasma 16SrII group. Comparisons between the species phylogeny and the phylogenies of the PMU genes revealed that the PnWB PMU is likely to have been transferred from the 16SrI group. This indicates that PMUs are not only the DNA unit for transposition within a genome, but also for horizontal transfer among divergent phytoplasma lineages. Given the association of PMUs with effector genes, the mobility of PMUs across genomes has important implications for phytoplasma ecology and evolution.

Keywords: composite transposon, effector, horizontal gene transfer, Mollicutes, phytoplasma, potential mobile unit
Abbreviations: AY-WB, aster yellows-witches’ broom; HGT, horizontal gene transfer; OY-M, onion yellows-mild; PnWB, peanut witches’ broom; PMU, potential mobile unit
Submitted: 06/19/13
Revised: 08/11/13
Accepted: 08/13/13
Citation: Ku C, Lo W-S, Kuo C-H. Horizontal transfer of potential mobile units in phytoplasmas. Mobile Genetic Elements 2013; 3:e26145; http://dx.doi.org/10.4161/mge.26145
*Correspondence to: Chih-Horng Kuo; Email: chk@gate.sinica.edu.tw

Commentary to: Chung W-C, Chen L-L, Lo W-S, Lin C-P, Kuo C-H. Comparative analysis of the peanut witches’ broom phytoplasma genome reveals horizontal transfer of potential mobile units and effectors. PLoS One 2013; 8:e62770; PMID:23626855; http://dx.doi.org/10.1371/journal.pone.0062770

Phytoplasmas: Insect-Transmitted Phytopathogens with Reduced Genomes

Phytoplasmas are a genus of wall-less bacteria that cause diseases in a wide variety of plant species, including many important crops and vegetables.1 Taxonomically, they are placed with the vertebrate-pathogenic mycoplasmas and the mostly arthropod-associated spiroplasmas within the class Mollicutes. Unlike these two other groups, phytoplasmas are hitherto uncultivated in vitro and are given the provisional status ‘Candidatus Phytoplasma’.2 In infected plants, they are mainly restricted to the enucleate sieve cells of the phloem and are transmitted to sap-feeding insect vectors in the order Hemiptera.3 Once acquired by the insects, phytoplasmas pass through the midgut, enter the hemocoel, and spread to various organs including the salivary gland, from which they are passed on to uninfected plants.4 Phytoplasmas not only utilize the host resources to replicate, but also manipulate the hosts to increase their own fitness. The key to the manipulations is effector (virulence) proteins secreted by phytoplasmas and targeted at molecules of host cells.5 For example, some effectors stimulate plant hosts to develop symptoms such as phyllody and virescence of flowers, generating more vegetative tissues where phytoplasmas reside.6 Some effectors can even alter the interactions between the eukaryotic hosts by reducing the defense responses of plants so that they become more vulnerable to phloem-feeding insects.7

To gain insights into the adaptations of these pathogens to the life within the dual eukaryotic hosts, the genomes of four phytoplasmas were completely sequenced, including the onion yellows-mild (OY-M)8 and aster yellows-witches’ broom (AY-WB)9 strains of ‘Ca. P. asteris’, ‘Ca. P. australiense’,10 and ‘Ca. P. mali’.11 It was found that phytoplasmas have reduced, repeat-rich genomes12 and that several of the putative effector genes are shared by the
common ancestor of the four phytoplasmas. However, based on the 16S rRNA gene phylogeny, where phytoplasmas are divided into three major clades, three of these four phytoplasmas (the two ‘Ca. P. asteris’ strains from the 16SrI group and ‘Ca. P. australiense’ from the 16SrXII group) are in Clade I and the other (‘Ca. P. mali’ from the 16SrX group) is in Clade III.16

Table 1. Genes present in the potential mobile unit (PMU) identified in the peanut witches’ broom phytoplasma genome

| Locus tag     | Gene name | Description                          |
|---------------|-----------|--------------------------------------|
| PnWB_v1c0470  | fltA      | DNA-directed RNA polymerase sigma factor |
| PnWB_v1c0480  | ssb       | single-stranded DNA-binding protein   |
| PnWB_v1c0495  | dam†       | adenine-specific DNA methyltransferase |
| PnWB_v1c0500  | himA      | DNA-binding protein HU               |
| PnWB_v1c0510  | hflB      | ATP-dependent Zn protease            |
| PnWB_v1c0520  | NA        | hypothetical protein                 |
| PnWB_v1c0530  | NA        | hypothetical protein                 |
| PnWB_v1c0540  | smc       | chromosome segregation ATPase-like protein |
| PnWB_v1c0550  | NA        | putative effector                    |
| PnWB_v1c0560  | NA        | hypothetical protein                 |
| PnWB_v1c0570  | NA        | phage-related protein                |
| PnWB_v1c0580  | tmk†       | thymidylate kinase                   |
| PnWB_v1c0600  | NA        | hypothetical protein                 |
| PnWB_v1c0610  | NA        | hypothetical protein                 |
| PnWB_v1c0620  | dnaB      | replicative DNA helicase             |
| PnWB_v1c0625  | gepA*      | phage-associated protein, fragment   |
| PnWB_v1c0630  | dnaG      | DNA primase                          |
| PnWB_v1c0640  | NA        | phase variable surface lipoprotein   |
| PnWB_v1c0650  | tra5      | putative transposase                 |

Potential Mobile Units are Transferred Among Phytoplasmas

The previously sequenced phytoplasma genomes all have large repetitive regions called potential mobile units (PMUs) that appear to have promoted intrachromosomal recombination. They are potentially mobile because they contain several core genes related to DNA recombination and replication, suggestive of the ability to transpose in a replicative manner within genomes. The PMUs are hypothesized to be composite transposons based on the presence of IS3 family transposase genes (tra5) and inverted repeats at the borders of the PMU.9 Experimental evidence further demonstrated that PMU1, the longest (~20 kb) and apparently the most complete PMU copy in AY-WB,9 has a self-replicating extrachromosomal circular form, suggesting PMUs can transpose through circular intermediates. Whereas PMU1 seems to have been derived from its circular form recently (or vice versa), the other AY-WB PMUs (PMU2-4) and PMU-like regions appear to be the degenerated versions of PMU1 and contain fewer or truncated open reading frames. This observation may be explained by the deletional bias observed in most bacterial genomes and the high levels of genetic drift experienced by obligate pathogens.

In our analysis of the PnWB phytoplasma draft genome assembly, one PMU region was identified (Fig. 1; Table 1). To investigate the relationships of this PMU with other phytoplasma PMUs, phylogenetic analyses were performed for homologs of three PMU signature genes (hflB, dnaB, and dnaG) from the PnWB PMU and all PMU copies in the other phytoplasmas. To our surprise, significant incongruence was found between these PMU gene trees and the species phylogeny. Based on either the 16S rRNA gene or the conserved single-copy protein-coding genes,
the PnWB phytoplasma and ‘Ca. P. mali’ form a monophyletic group. However, the three PMU gene trees all support a close relationship between the PnWB PMU and homologs in the ‘Ca. P. asteris’ OY-M genome. The most plausible explanation for this observation is that the PnWB PMU originated from one of the PMUs present in a close relative of OY-M. This horizontal gene transfer (HGT) is likely to have occurred after the divergence of OY-M and AY-WB, which is relatively recent given the short branch lengths between these two strains of ‘Ca. P. asteris’ in the species phylogeny.16 The agreement between these two strains of OY-M and AY-WB, which is relatively horizontal gene transfer (HGT) is likely present in a close relative of OY-M. This PMU originated from one of the PMUs for this observation is that the PnWB phytoplasma strains can co-occur in the same plant host24 or insect vector4 and the interactions among these strains could influence their virulence to the host25 and the transmission efficiency of the individual strains.26 The results from our PnWB phytoplasma genome analysis further indicate that PMUs could be transferred among co-occurring phytoplasmas. Although DNA transfer among mollicutes has not been studied in detail, there is evidence in support of mechanisms that require cell contact (conjugation or cell fusion) in spiroplasmas27 and mycoplasmas.28 It has also been suggested that hypothetical genes on PMU1 that were predicted to be membrane-targeted may form plasma membrane-associated structures that allow transfer of the circular PMUs.12 As a prerequisite for HGT of PMUs, different phytoplasma strains must infect the same plant host or insect vector. This indicates that strains with a wider range of hosts (plant-generalist) or vectors (vector-generalist)26 would have a higher rate of donating their own PMUs to or receiving PMUs from other strains. In agreement with this, the putative donor of the PnWB PMU is ‘Ca. P. asteris’, which has been isolated from more than 80 plant species worldwide and are transmitted by 30 polyphagous insect vectors.3

Probably the most significant implication about PMU transfers is that effector genes can take free rides. In ‘Ca. P. asteris’, the majority of the effector genes are located in PMUs.12 The transfer of PMUs could help to spread the effector genes across different lineages of phytoplasmas. Since several effector proteins have been found to be effective against hosts in divergent lineages (e.g., Arabidopsis, Nicotiana),5,7 acquisition of new effectors may expand the host ranges of recipient strains, thus making the phytoplasma clade one of the most successful groups of phytopathogens. Although there are only one PMU identified in the PnWB genome sequence16 and one PMU (as well as a highly reduced PMU region) in ‘Ca. P. mali’,11 the numbers of putative effectors in these two genomes are comparable to those in the other three.16 This observation can be explained by the degeneration of integrated PMUs over time, as inferred from the reduced versions of PMUs or PMU-like regions in ‘Ca. P. asteris’.9,17 Effector genes may have been brought into the PnWB phytoplasma and ‘Ca. P. mali’ through PMU-mediated HGT, but with occurrences of random mutations, the PMU regions that are not maintained by natural selection gradually became degenerated. Eventually, most of the ancient PMUs became unrecognizable, while only the effector genes are retained due to the selective advantages they conferred. In contrast to the PnWB phytoplasma and ‘Ca. P. mali’, recent transposition activity in ‘Ca. P. asteris’ and ‘Ca. P. australiensis’ seem to have resulted in multiple PMU copies that account to a large part for the genome size differences among these phytoplasmas.9,11

Ecological Implications

It has been shown that different phytoplasma strains can co-occur in the same plant host24 or insect vector4 and the interactions among these strains could influence their virulence to the host25 and the transmission efficiency of the individual strains.26 The results from our PnWB phytoplasma genome analysis further indicate that PMUs could be transferred among co-occurring phytoplasmas. Although DNA transfer among mollicutes has not been studied in detail, there is evidence in support of mechanisms that require cell contact (conjugation or cell fusion) in spiroplasmas27 and mycoplasmas.28 It has also been suggested that hypothetical genes on PMU1 that were predicted to be membrane-targeted may form plasma membrane-associated structures that allow transfer of the circular PMUs.12 As a prerequisite for HGT of PMUs, different phytoplasma strains must infect the same plant host or insect vector. This indicates that strains with a wider range of hosts (plant-generalist) or vectors (vector-generalist)26 would have a higher rate of donating their own PMUs to or receiving PMUs from other strains. In agreement with this, the putative donor of the PnWB PMU is ‘Ca. P. asteris’, which has been isolated from more than 80 plant species worldwide and are transmitted by 30 polyphagous insect vectors.3

Probably the most significant implication about PMU transfers is that effector genes can take free rides. In ‘Ca. P. asteris’, the majority of the effector genes are located in PMUs.12 The transfer of PMUs could help to spread the effector genes across different lineages of phytoplasmas. Since several effector proteins have been found to be effective against hosts in divergent lineages (e.g., Arabidopsis, Nicotiana),5,7 acquisition of new effectors may expand the host ranges of recipient strains, thus making the phytoplasma clade one of the most successful groups of phytopathogens. Although there are only one PMU identified in the PnWB genome sequence16 and one PMU (as well as a highly reduced PMU region) in ‘Ca. P. mali’,11 the numbers of putative effectors in these two genomes are comparable to those in the other three.16 This observation can be explained by the degeneration of integrated PMUs over time, as inferred from the reduced versions of PMUs or PMU-like regions in ‘Ca. P. asteris’.9,17 Effector genes may have been brought into the PnWB phytoplasma and ‘Ca. P. mali’ through PMU-mediated HGT, but with occurrences of random mutations, the PMU regions that are not maintained by natural selection gradually became degenerated. Eventually, most of the ancient PMUs became unrecognizable, while only the effector genes are retained due to the selective advantages they conferred. In contrast to the PnWB phytoplasma and ‘Ca. P. mali’, recent transposition activity in ‘Ca. P. asteris’ and ‘Ca. P. australiensis’ seem to have resulted in multiple PMU copies that account to a large part for the genome size differences among these phytoplasmas.9,11

Future Perspectives

The presence of PMUs is a unique characteristic of the phytoplasma genomes characterized to date. The association of PMUs with effector genes and their ability to duplicate, transpose, and transfer across genomes may have contributed to the adaptation of phytoplasmas to their pathogenic lifestyle. However, the origin, diversity, and dynamics of PMUs in phytoplasma genomes remain unclear. To this end, two strategies for sampling of phytoplasma strains are required. At a deeper divergence level, genome sequences from strains that improve the taxon sampling of the entire phytoplasma clade are needed. These sequences may contain previously unknown types of PMUs like the PMU1-4 of ‘Ca. P. australiensis’9,16 and comparisons of the different types of PMUs may reveal the unifying characteristic of PMUs that holds clues to their origin. In addition, phylogenetic analyses of a more comprehensive sample of phytoplasma PMUs could shed light on the extent of HGT of PMUs across phytoplasma lineages. At the population level, genome sequencing for closely related strains of certain phytoplasma groups could provide insights into the evolutionary dynamics of PMUs at a shorter timescale. The presently available genome sequences, including those from the two strains of ‘Ca. P. asteris’,

©2013 Landes Bioscience. Do not distribute.
are too divergent for the inference of PMU dynamics in phytoplasma genomes. Through comparisons between genomes of closely related strains, either naturally isolated or laboratory-propagated, we may have glimpses of how PMUs integrate, recombine, and degenerate.

To meet the need for these phytoplasma genome sequences, high-throughput next-generation sequencing technologies hold great promise and have already been applied to sequencing several mollicutes genomes.16,21,23 One complicating factor for sequencing phytoplasma genomes is that DNA of these obligate pathogens is extracted along with the plant DNA, which is often that of the experimental host periwinkle (Catharanthus roseus).10,11,16 This problem would become more severe given that most next-generation sequencing platforms generate reads of shorter lengths than those of conventional Sanger sequencing. One strategy to improve the quality of assembly with reads from multiple sources would be to filter out the reads from undesirable sources.29 For this purpose, the recently available periwinkle chloroplast genome sequence may be utilized to filter out reads stemming from the chloroplast genome, which often comprises a large portion of the plant DNA and has a low GC content like that of phytoplasmas.30

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
1. Bertaccini A, Duduk B. Phytoplasma and phytoplasma diseases: a review of recent research. Phytopathol Meditter 2009; 48:355-78
2. IRPCM Phytoplasma/Spiroplasma Working Team.-Phytoplasma Taxonomy Group. 'Candidatus Phytoplasma aurantifolia' a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. Int J Syst Evol Microbiol 2004; 54:1243-55; PMID:15280299; http://dx.doi.org/10.1099/ijs.0.02854-0
3. Hogenhout SA, Oshima K, Ammar D, Kakizawa S, Kingdom HN, Nambara S. Mollicutes: bacteria that manipulate plants and insects. Mol Plant Pathol 2008; 9:403-23; PMID:18705857; http://dx.doi.org/10.1111/j.1364-5703.2008.00472.x
4. Weintraub PG, Beanland L. Insect vectors of phytoplasmas. Annu Rev Entomol 2006; 51:91-111; PMID:16332205; http://dx.doi.org/10.1146/annurev.ento.51.110104.151039
5. Sugio A, MacLean AM, Kingdom HN, Grieve VM, Manimekalai R, Hogenhout SA. Diverse targets of phytoplasma effectors: from plant development to defense against insects. Annu Rev Phytopathol 2011; 49:175-95; PMID:21838574; http://dx.doi.org/10.1146/annurev-phyto-072010-095323
6. MacLean AM, Sugio A, Makarova OV, Findlay KC, Grieve VM, Töth R, Nicolaïsm M, Hogenhout SA. Phytoplasma effector SAP54 induces indeterminate leaf-like flower development in Arabidopsis plants. Plant Physiol 2011; 157:831-41; PMID:21849514; http://dx.doi.org/10.1104/pp.111.185586
7. Sugio A, Kingdom HN, MacLean AM, Grieve VM, Hogenhout SA. Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. Proc Natl Acad Sci U S A 2011; 108:E1254-63; PMID:22065743; http://dx.doi.org/10.1073/pnas.1005664108
8. Oshima K, Kakizawa S, Nishigawa H, Jung H-Y, Wei W, Suzuki S, Arashida R, Nakata D, Miyata S, Ugaki M, et al. Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma. Nat Genet 2004; 36:27-9; PMID:14661021; http://dx.doi.org/10.1038/ng.1038
9. Bai X, Zhang J, Ewing A, Miller SA, Jancso Radek A, Gundersen D, et al. Gene-based phytopathogenic classification of 'Candidatus phytoplasma mali' strains and evidence that strain composition determines virulence in multiply infected apple trees. Mol Plant Microbe Interact 2011; 24:1258-66; PMID:21899439; http://dx.doi.org/10.1094/MPMI-05-11-0126
10. Bosco D, D'Amelio R. Trismisomics and specificity of multiple phytoplasmas in the insect vector. In: Weintraub PG, Jones P, eds. Phytoplasmas Genomes, Plant Hosts and Vectors. Oxfordshire: CAB, 2010:293-308
11. Kuo C-H, Oehmen H. Deleterious bias across the three domains of life. Genome Biol Evol 2009; 1:145-52; PMID:20333185; http://dx.doi.org/10.1093/gbe/evp016
12. Kuo-C-H, Moran NA, Oehmen H. The consequences of genomic drift for bacterial genome complexity. Genome Res 2009; 19:1450-4; PMID:19502381; http://dx.doi.org/10.1101/gr.097858.109
13. Wei W, Davis RE, Lomatiene R, Zhao Y, Ancient, recurrent phage attacks and recombination shaped host periwinkle genome evolution. Proc Natl Acad Sci USA 2008; 105:11827-32; PMID:18705857; http://dx.doi.org/10.1073/pnas.0805237105
14. Ku C, Lo WS, Chen L-L, Kuo C-H. Complete genomes of two dipetalon-associated phytoplasmas provided insights into the origin, dynamics, and impacts of viral invasion in 'Spiroplasma citri'. Genome Biol Evol 2013; 5:1151-64; PMID:23711669; http://dx.doi.org/10.1093/gbe/evt084