*Bulletin of Insectology* **64** *(Supplement): S9-S11, 2011*
ISSN 1721-8861

# Insights in host dependency encoded within phytoplasma genomes

**Michael Kube**[1,2]
[1]*Max Planck Institute for Molecular Genetics, Berlin, Germany*
[2]*Humboldt Universität zu Berlin, Institute for Horticultural Sciences, Department of Phytomedicine, Berlin, Germany*

## Abstract

A strong community of scientists has been established within the last 40 years in phytoplasma research. Hereby, knowledge of these phytopathogenic bacteria increased dramatically in all fields catalysed by the introduction of molecular techniques and bioinformatics. The determination and analysis of genome data provided insights in the genome organization, content, evolution but also an excellent database for experiments studying putative virulence factors and for the development of diagnostics tools. In contrast to this progress, the understanding of the metabolism appears still weak. Its interpretation is essential for the understanding of the pathogen-host and host-pathogen interaction. An overview about the genetic information in the '*Candidatus* Phytoplasma asteris' strains OY (line OY-M), AY-WB, '*Ca.* P. australiense', and '*Ca.* P. mali' is provided with respect to the encoded pathways and the resulting general requirements on the host.

**Key words:** comparative genomics, sieve cell sap, metabolism.

## Introduction

Phytoplasma research blooms since the assignment of these fascinating phytopathogenic organisms as bacteria in 1967 (Doi *et al.*, 1967). In contrast to many other *Mollicutes*, it was not possible to cultivate phytoplasmas in cell-free media so far. This fact is severely handicapping phytoplasma research and in particular the experimental analysis of the metabolic capacities. Molecular analysis of phytoplasma genes and genomes is thus an essential means to improve our knowledge of diagnosis, evolution, pathogenicity and metabolism of these bacteria.

## Genomes of phytoplasmas

The complete determination of four phytoplasma genomes including those of '*Ca.* Phytoplasma asteris' strains OY (line OY-M) and AY-WB (Oshima *et al.*, 2004; Bai *et al.*, 2006), '*Ca.* P. australiense' (Tran-Nguyen *et al.*, 2008) and '*Ca.* P. mali' (Kube *et al.*, 2008) provided valuable insights into the genetic environment of these bacteria. These strains represent only a relatively small part of phytoplasma diversity as we know it today. Sequence information provided significant insights in chromosome organization, such as the circular and linear forms and the variation in genome size (Neimark *et al.*, 1993). Within the last years it has become clear that this variation is mainly based on integration and duplication events. The integration of complex transposons (PMUs) (Bai *et al.*, 2006; Toruno *et al.*, 2010), prophage-related elements (Wei *et al.*, 2008), and plasmid sequences (Bai *et al.*, 2006; Liefting *et al.*, 2006) results in genome instability (Bai *et al.*, 2006), an increase of the genome size (Bai *et al.*, 2006; Kube *et al.*, 2008), and stands in contrast to genome condensation processes. The importance of these events for virulence remains unclear due to the limited number of virulence-related genes identified so far. An indirect effect is probably given for the extrachromosomal elements because it was shown that their genetic material influences vector transmissibility at least of some strains (Ishii *et al.*, 2009). Examples of integration events can be identified in each phytoplasma genome examined so far. The genome data suggest that the integrated genetic material is, on one hand, of significant importance for the phytoplasmas but, on the other hand, probably also an accident resulting from a high pressure of integration events.

## Metabolism and requirements on the host

The impact of these genetic elements on the limited chromosomal metabolic capabilities of phytoplasmas appears to be low. The metabolic repertoire deduced from the four phytoplasma chromosomes is characterized by the loss of pathways and obviously corresponds to an adaptation to plant and insect hosts. Genome condensation resulted in the absence of the F0F1 ATPase system in all four phytoplasma genomes. A complete gene set encoding all proteins needed for the glycolysis was identified in phytoplasmas except for '*Ca.* P. mali', which is lacking the energy yielding part. This finding raises the question, which alternative pathway is encoded in the phytoplasmas to gain ATP (Kube *et al.*, 2008). Phytoplasmas lack the gene sets for the sterol biosynthesis, tricarboxylic acid cycle, phosphotransferase system, nucleotide *de novo* synthesis and amino acid synthesis. These deficiencies result in strong requirements on the plant host and the colonized environment of the sieve tubes for example. The sieve tube sap must and can provide a wide variety of metabolites needed in consequence. Its composition varies depending on the plant species, but always contains large amounts of carbohydrates. The most abundant is sucrose beside other sugars, minerals, proteins, amino acids, ATP (van Helden *et al.*, 1994; van Bel and Hess, 2008) and usually low amounts of bicarbonate and malate. Malate and citrate represent the predominant organic acids in phloem and xylem sap (Ziegler, 1975). Phytoplasmas share a common genetic repertoire for the uptake of the components which they are incapable to synthetize and for the cofactors needed for their functional protein machinery. The complete pathways are limited to replication, repair, transcription, translation and carbohydrate metabolism. In particular, the metabolism differs with respect to the phylogenetic branch and still appears incomplete with respect to our current knowledge. However, besides the differences in the genome size and the number of paralogs, all phytoplasmas share a remarkable common set of protein functions.

## Membrane proteins and secretion

Immunodominant membrane proteins represent the major portion of the total cellular membrane proteins in most phytoplasmas (Kakizawa *et al*., 2006a) and probably interact with the insect host at least (Suzuki *et al.*, 2006; Kakizawa *et al*., 2006b). The percentage of predicted proteins by Phobius (Käll *et al.*, 2004) carrying transmembrane helices ranges from 26-31 % of the annotated genes. ATPases involved in cation exchange and mechanosensitive channel proteins were embedded in the membrane of all four phytoplasmas in addition to the ABC transporters and symporter required to supply the phytoplasma cytosol with the essential biochemical modules. A functional active sec-dependent secretion system works in the opposite direction and allows the export of signal peptide carrying proteins (figure 1). Around 5% of all proteins belongs to this group including prominent members such as SAP11 (Bai *et al.*, 2009) and the protein 'tengu' of OY-M (Hoshi *et al.*, 2009), which induces witches' broom and dwarfism.

## Outlook

Other proteins involved in manipulation of the plant and/or insect host will be identified by genetic manipulation, genome comparison and expression studies within the next years. Impact in phytoplasma research will also be provided by studies of the analysis of metabolism and the corresponding release of metabolic products and their so far unknown impact in virulence. These analyses will be supported by experimental studies of the genome, transcriptome and proteome. New high throughput techniques within these research areas, such as deep sequencing by next generation sequencing, allow the determination of phytoplasma and the discrimination or analysis of host derived data. First projects are completed (Ji *et al.*, 2009) and several others are in progress worldwide. Results are needed for deeper insights in these parasitic bacteria.

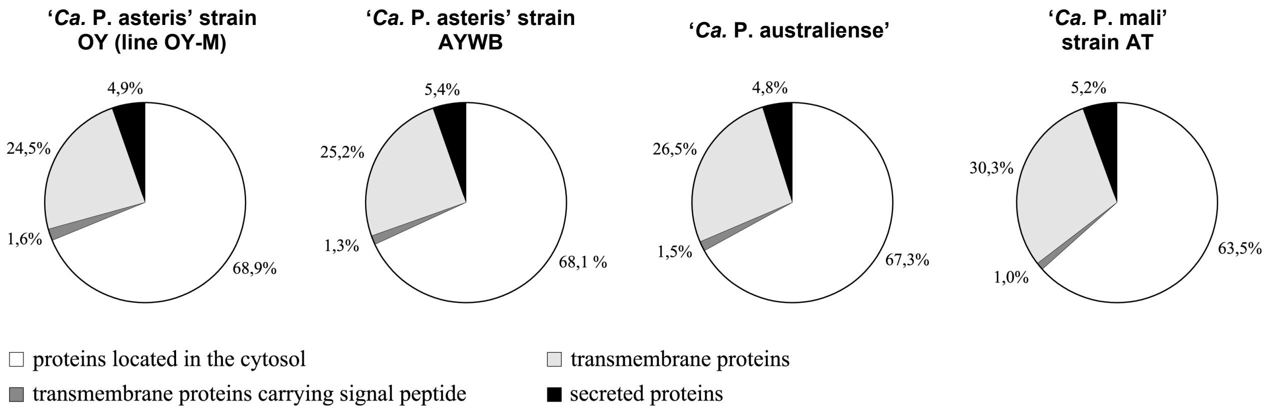

**Figure 1.** Percentage of the putative secreted proteins calculated on the deduced proteins of the chromosomes (AP006628.2, CP000061, AM422018 and CU469464).

## Acknowledgements

I am grateful to Jelena Mitrovic, Toni Luge, Andreas Stier and Erich Seemüller. This study was supported by the Max Planck Society.

## References

Bai X., Zhang J., Ewing A., Miller S. A., Radek J. A., Shevchenko D. V., Tsukerman K., Walunas T., Lapidus A., Campbell J. W., Hogenhout S. A., 2006.- Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts.- *The Journal of Bacteriology*, 188: 3682-3696.

Bai X., Correa V. R., Toruño T. Y., Ammar el-D., Kamoun S., Hogenhout S. A., 2009.- AY-WB phytoplasma secretes a protein that targets plant cell nuclei.- *Molecular Plant-Microbe Interactions*, 22: 18-30.

Doi Y., Teranaka M., Yora K., Asuyama H., 1967.- Mycoplasma-or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom.- *Annals of the Phytopathological Society of Japan*, 33: 259-266.

Hoshi A., Oshima K., Kakizawa S., Ishii Y., Ozeki J., Hashimoto M., Komatsu K., Kagiwada S., Yamaji Y., Namba S., 2009.- A unique virulence factor for proliferation and dwarfism in plants identified from a phytopathogenic bacterium.- *Proceedings of the National Academy of Sciences of the United States of America*, 106: 6416-6421.

Ishii Y., Oshima K., Kakizawa S., Hoshi A., Maejima K., Kagiwada S., Yamaji Y., Namba S., 2009.- Process of reductive evolution during 10 years in plasmids of a non-insect-transmissible phytoplasma.- *Gene*, 446: 51-57.

Ji X., Gai Y., Zheng C., Mu Z., 2009.- Comparative proteomic analysis provides new insights into mulberry dwarf responses in mulberry (*Morus alba* L.).- *Proteomics*, 9: 5328-5339.

Kakizawa S., Oshima K., Jung H. Y., Suzuki S., Nishigawa H., Arashida R., Miyata S., Ugaki M., Kishino H., Namba S., 2006a.- Positive selection acting on a surface membrane protein of the plant-pathogenic phytoplasmas.- *The Journal of Bacteriology*, 188: 3424-3428.

Kakizawa S., Oshima K., Namba S., 2006b.- Diversity and functional importance of phytoplasma membrane proteins.- *Trends in Microbiology*, 14: 254-265.

Käll L., Krogh A., Sonnhammer E. L. L., 2004.- A combined transmembrane topology and signal peptide prediction method.- *Journal of Molecular Biology*, 338: 1027-1036.

Kube M., Schneider B., Kuhl H., Dandekar T., Heitmann K., Migdoll A.M., Reinhardt R., Seemüller E., 2008.- The linear chromosome of the plant-pathogenic mycoplasma '*Candidatus* Phytoplasma mali'.- *BMC Genomics*, 9: 306.

Liefting L. W., Andersen M. T., Lough T. J., Beever R. E., 2006.- Comparative analysis of the plasmids from two isolates of '*Candidatus* Phytoplasma australiense'.- *Plasmid*, 56: 138-144.

Neimark H., Kirkpatrick B. C., 1993.- Isolation and characterization of full-length chromosomes from non-culturable plant-pathogenic mycoplasma-like organisms.- *Molecular Microbiology*, 7: 21-28.

Oshima K., Kakizawa S., Nishigawa H., Jung H. Y., Wei W., Suzuki S., Arashida R., Nakata D., Miyata S., Ugaki M., Namba S., 2004.- Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma.- *Nature Genetics*, 36: 27-29.

Suzuki S., Oshima K., Kakizawa S., Arashida R., Jung H. Y., Yamaji Y., Nishigawa H., Ugaki M., Namba S., 2006.- Interaction between the membrane protein of a pathogen and insect microfilament complex determines insect-vector specificity.- *Proceedings of the National Academy of Sciences of the United States of America*, 103: 4252-4257.

Toruño T. Y., Musić M. S., Simi S., Nicolaisen M., Hogenhout S. A., 2010.- Phytoplasma PMU1 exists as linear chromosomal and circular extrachromosomal elements and has enhanced expression in insect vectors compared with plant hosts.- *Molecular Microbiology*, 77: 1406-14015.

Tran-Nguyen L. T., Kube M., Schneider B., Reinhardt R., Gibb K. S., 2008.- Comparative genome analysis of '*Candidatus* Phytoplasma australiense' (subgroup tuf-Australia I; rp-A) and '*Ca.* P. asteris' strains OY-M and AY-WB.- *The Journal of Bacteriology*, 190: 3979-3991.

van Bel A. J., Hess P. H., 2008.- Hexoses as phloem transport sugars: the end of a dogma?- *Journal of Experimental Botany*, 59: 261-272.

van Helden M., Tjallingh W. F., van Beek T. A., 1994.- Phloem sap collection from lettuce (*Lactuca sativa* L.): Chemical comparison among collection methods.- *Journal of Chemical Ecology*, 20: 3191-3206.

Wei W., Davis R. E., Jomantiene R., Zhao Y., 2008.- Ancient, recurrent phage attacks and recombination shaped dynamic sequence-variable mosaics at the root of phytoplasma genome evolution.- *Proceedings of the National Academy of Sciences of the United States of America*, 105: 11827-11832.

Ziegler H. 1975.- Nature of substances in phloem, pp. 57-100. In: *Encyclopedia of plant physiology. Vol. 1, transport in plants* (Zimmermann M. H., Milburn J. A., Eds).- Springer-Verlag, Berlin, Germany.

**Corresponding author:** Michael Kube (e-mail: kube@molgen.mpg.de), Max Planck Institute for Molecular Genetics, Ihnestr. 63-73, 14195 Berlin, Germany.