Bulletin of Insectology ***60*** *(2): 111-112, 2007*
ISSN 1721-8861

An overview of the genome sequence of '*Candidatus* Phytoplasma australiense' – Australian strain

Lucy TRAN-NGUYEN[1,4], Michael KUBE[2], Bernd SCHNEIDER[3], Richard REINHARDT[2], Karen GIBB[1]
[1]*Charles Darwin University, Darwin, Australia*
[2]*Max Planck Institute for Molecular Genetics, Berlin, Germany*
[3]*Federal Biological Research Institute for Agriculture and Forestry, Dossenheim, Germany*
[4]*Department of Primary Industry, Fisheries and Mines, Darwin, Australia*

Abstract

The chromosome sequence of '*Candidatus* Phytoplasma australiense' was determined. The circular chromosome comprised of ~880 kb with a GC content of 27% containing 839 predicted ORFs.

Initial comparative analysis of '*Ca.* P. australiense' with '*Ca.* Phytoplasma asteris' strains OY-M and AY-WB showed that the gene order was more conserved between the closely related '*Ca.* P. asteris' strains than to '*Ca*. P. australiense'. Prominent differences observed between '*Ca.* P. australiense' and '*Ca.* P. asteris' strains included the genome size (about 19 kb larger than OY-M); encoded the largest number of genes with assigned functions and hypothetical proteins with unknown functions.

Key words: '*Candidatus* Phytoplasma australiense', genome sequence, comparative analysis.

Introduction

Phytoplasmas are bacterial plant pathogens in the class *Mollicutes* that are associated with over 1,000 plant diseases worldwide (Seemüller *et al*., 1998; Lee *et al*., 2000). Phytoplasmas have genome sizes between 530 and 1,200 kb, lack an outer cell-wall, the G + C content ranges between 23 and 29 mol%, they possess two rRNA operons, have a low number of tRNAs and a limited set of metabolic enzymes (Bové, 1997; Marcone *et al*., 1999; Bai *et al*., 2006; Oshima *et al*., 2004).

In Australia, '*Candidatus* Phytoplasma australiense' is widespread and associated with several diseases in economically important crops. These diseases include Australian grapevine yellows (AGY; Padovan *et al*., 1995), papaya dieback (PDB; Liefting *et al*., 1998), strawberry lethal yellows (SLY), strawberry green petal (SGP; Padovan *et al*., 2000) and pumpkin yellow leaf curl (PYLC; Streten *et al.,* 2005).

Mollicutes have been targets for genome sequencing projects due to their small genomes and economic importance in plant and animal diseases. Whole genome projects provide insight into the organism's biology such as the minimal gene set for survival; nutritional requirements; energy metabolism to understand pathogenicity; and nucleic acid metabolism to understand host-pathogen interactions (Firrao *et al*., 2007).

To date, 16 mollicute genomes have been fully sequenced (http://cbi.labri.fr/outils/molligen/home.php) including two phytoplasmas, '*Ca*. P. asteris' strains onion yellows mutant (OY-M; Oshima *et al*., 2004) and aster yellows witches' broom (AY-WB; Bai *et al*., 2006). Information derived from the two phytoplasma genomes include features such as reduced metabolic functions compared to mycoplasmas; absence of the pentose phosphate cycle; no ATP synthase subunits; repeated DNA organised in potential mobile units (Oshima *et al*., 2004; Bai *et al*., 2006)

Materials and methods

'*Ca.* P. australiense' was transmitted from *Gomphocarpus physocarpus* (Cottonbush**)** QLD, Australia to periwinkle by grafting. The phytoplasma strain was maintained in an insect-proof glasshouse in periwinkle by periodic grafting.

Periwinkle flowers were used as a DNA source for pulsed field gel electrophoresis (PFGE). The unstained chromosomal DNA was electroeluted from the excised PFGE agarose slice and concentrated by ethanol precipitation using glycogen as a carrier. Two shotgun libraries were generated from sonicated DNA.

Sequences were assembled using Phrap and the Consed package (version 14.00). Gaps and regions of poor sequence quality were improved by resequencing, primer walking and long-range PCR. Annotation was achieved using High Throughput Gene Annotation (HTGA) at the Max Planck Institute.

Comparative genome analysis was conducted using Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Molligen database.

Results

The '*Ca.* P. australiense' genome is comprised of one circular chromosome of 880 kb and contains two rRNA operons, 35 tRNAs, two miscellaneous RNA genes and 839 predicted ORFs with a minimal size of 30 amino acid residues.

Initial comparative genome analysis showed that all three phytoplasma genomes were missing complete pathways for amino- and nucleotide sugars, glyoxylate and dicarboxylate metabolism (carbohydrate metabolism); ATP synthesis; fatty acid metabolism and carbon dioxide fixation (energy and lipid metabolism); phenylalanine metabolism; the urea cycle and metabo-

lism of amino groups; D-glutamine, D-glutamate, D-arginine, D-ornithine, D-alanine and D-glutathione metabolism (amino acid metabolism); type I and II secretion pathways, the phosphoenolpyruvate-dependent phosphotransferase system (PTS) involved in membrane transport and the two-component system involved in signal transduction.

Discussion

Phytoplasmas and *Mollicutes* in general have distinctive genomic features such as reduced genomes, low GC content, encode fewer than 1,000 genes and have limited metabolic capability. Whole genome sequence should elucidate the pathogen's biology, how it interacts with its host and its metabolic capabilities.

'*Ca*. P. australiense' metabolic pathways are similar to those of OY-M and AY-WB. The missing PTS system in phytoplasmas sets them apart from those in the SEM clade since they are unable to import sugars using the multiprotein system. Phytoplasmas instead may rely on their ABC transporters to import sugars (Bai *et al*., 2006).

The number of homologous genes between the two '*Ca*. P. asteris' strains, and '*Ca*. P. australiense' strain, is indicative of the relationship of the strains. Based on the similarity of genes, the OY-M and AY-WB phytoplasmas were more closely related to each other than they were to '*Ca*. P. australiense'.

Acknowledgements

This study was supported by the Max Planck Society, the Federal Biological Research Centre for Agriculture and Forestry (BBA), and funded by the former CRC for Tropical Plant Protection and Charles Darwin University. The CRC for National Plant Biosecurity for funding L. Tran-Nguyen's trip to attend the First International Phytoplasma Working Group Meeting.

References

BAI X., ZHANG J., EWING A., MILLER S.A., RADEK A., SCHEVCHENKO D., 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.- *Journal of Bacteriology,* 188 (10): 3682-3696.

BOVÉ J. M., 1997.- Spiroplasmas: infectious agents of plants, arthropods and vertebrates.- *Wiener klinische Wochenschrift*, 109: 604-612.

FIRRAO G., GARCIA-CHAPA M., MARZACHI C., 2007.- Phytoplasmas: genetics, diagnosis and relationships with the plant and insect host.- *Frontiers in Bioscience*, 12: 1353-1375.

LEE I.-M., DAVIS R. E., GUNDERSEN-RINDAL D. E., 2000.- Phytoplasmas: phytopathogenic mollicutes.- *Annual Review of Microbiology,* 54: 221-255.

LIEFTING L. W., PADOVAN A. C., GIBB K. S., BEEVER R. E., ANDERSEN M. T., NEWCOMB R. D., BECK D. L., FORSTER R. L. S., 1998.- '*Candidatus* Phytoplasma australiense' is the phytoplasma associated with Australian grapevine yellows, papaya dieback, and *Phormium* yellow leaf diseases.- *European Journal of Plant Pathology*, 104: 619-623.

MARCONE C., NEIMARK H., RAGOZZINO A., LAUER U., SEEMÜLLER E., 1999.- Chromosome sizes of phytoplasmas composing major phylogenetic groups and subgroups.- *Phytopathology*, 89 (9): 805-810.

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.

PADOVAN A. C., GIBB K. S., BERTACCINI A., VIBIO M., BONFIGLIOLI R. E., MAGAREY,P. A., SEARS B. B., 1995.- Molecular detection of the Australian Grapevine Yellows phytoplasma and comparison with grapevine yellows phytoplasmas from Italy.- *Australian Journal of Grape and Wine Research*, 1: 25-31.

PADOVAN A. C., FIRRAO G., SCHNEIDER B., GIBB K. S., 2000.- Chromosome mapping of the sweet potato little leaf phytoplasma reveals genome heterogeneity within the phytoplasmas.- *Microbiology,* 146: 893-902.

SEEMÜLLER E., MARCONE C., LAUER U., RAGOZZINO A., GOSCHL M., 1998.- Current status of molecular classification of the phytoplasmas.- *Journal of Plant Pathology*, 80: 3-26.

STRETEN C., CONDE B., HERRINGTON M., MOULDEN J., GIBB K., 2005.- '*Candidatus* Phytoplasma australiense' is associated with pumpkin yellow leaf curl disease in Queensland, Western Australia and the Northern Territory.- *Australasian Plant Pathology*, 34: 103-105.

Corresponding author: Lucy TRAN-NGUYEN (e-mail: lucy.tran-nguyen@nt.gov.au), Diagnostic Services Division, DPIFM, Darwin, Australia.