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First look into the genome sequence of '*Candidatus* Phytoplasma mali' in comparison to '*Candidatus* Phytoplasma asteris' strains OY-M and AY-WB

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Abstract

The genome of '*Candidatus* Phytoplasma mali' (strain AT) was determined, it represents the smallest phytoplasma genome sequenced so far. As in the other phytoplasmas, the metabolic capabilities are strongly reduced and reflect the gene repertoire of a parasitic organism. The chromosome is characterized by large repetitive elements and a low content of minor repeats. Preliminary comparative analyses of the genome of '*Ca.* P. mali' (strain AT) clearly indicate a distinct position and probably a higher level of genome reduction compared to the aster yellows phytoplasmas.

Key words: '*Candidatus* Phytoplasma mali', strain AT, genome characteristics, comparative analysis.

Introduction

'*Candidatus* Phytoplasma mali' is inducing apple proliferation (AP), one of the economically most important phytoplasma diseases in Europe. The causal agent is a minute, wall-less and phloem-residing bacterium that is transmitted from plant to plant by two psyllids, *Cacopsylla picta* (Foerster) and *Cacopsylla melanoneura* (Foerster).

'*Ca.* P. mali' forms, together with *'Ca.* P. pyri' and '*Ca.* P. prunorum', that are causing pear decline and European stone fruit yellows, respectively, and a few other phytoplasmas, a major subclade in phytoplasma phylogenetic tree (Seemüller *et al.*, 2002). In order to better understand pathogenicity and metabolic capacity of the yet uncultivable phytoplasmas, the genomes of four phytoplasmas have been fully sequenced (Bai *et al.*, 2006; Liefting *et al.*, 2006; Oshima *et al.*, 2004; Tran-Nguyen *et al.*, 2006). The four phytoplasmas include strains OY and AY-WB of the aster yellows (AY) group represented by '*Ca.* P. asteris 'and a New Zealand and an Australian strain of '*Ca.* P. australiense'. These two putative species differ little from each other at the 16S rDNA level and are members of the same major phytoplasma subclade that is different from that the fruit tree phytoplasmas belong to (Seemüller *et al.*, 2002).

Here we present first insights into the '*Ca.* P. mali' genome.

Materials and methods

'*Ca.* P. mali' was transmitted from a symptomatic apple tree to various *Nicotiana tabacum* cultivars from which chromosomal DNA was prepared. Genome sequencing was performed by a whole genome shotgun approach, based on plasmid libraries (Kube *et al.*, 2005). Sequence assembly was performed using PHRAP (www.phrap.org) and Consed (Gordon *et al.*, 2004). A prediction of tRNA and rRNAs was done using tRNAscan-SE (Lowe and Eddy, 1997) and Rfam (Griffiths-Jones, 2005) followed by an automated functional annotation with the High Throughput Gene Annotation (HTGA) platform (Kube *et al.*, 2005) at the Max Planck Institute. Verification of the genomic sequence, control of results from the automated ORF prediction and their functional assignment are still in progress.

Preliminary results

The genome of '*Ca.* P. mali' (strain AT) was determined to be the smallest phytoplasma genome sequenced so far. Preliminary analyses of the chromosome with a total length of about 600 kb show the complete set of tRNAs and ribosomal operons. An automated annotation (HTGA) results in a functional assignment of about 300 ORFs. About one third of the predicted ORFs were classified as coding for hypothetical or conserved hypothetical proteins. The overall coding capacity is estimated of ~78%. As in the other phytoplasmas the metabolic capabilities are strongly reduced and reflect the gene repertoire of a parasitic organism. About 100 paralogous genes were identified mostly coding for hypothetical or membrane transport proteins. Large repetitive elements exist and a few minor repeats are dispersed within the chromosome.

The GC content is well below the one of the aster yellows phytoplasmas OY-M and AY-WB, with 28 and 27 mol% GC, respectively. The chromosome of '*Ca.* P. mali' strain AT contains about 340 and 250 ORFs less than OY-M and AY-WB, respectively. The percentage of hypothetical genes versus all genes is similar to AY-WB, but less than for OY-M. First results also indicate that '*Ca.* P. mali' strain AT lacks a series of key enzymes in the carbohydrate metabolism present in the aster yellows phytoplasmas but possess a few sporadic enzymes not present in OY-M and AY-WB.

Discussion

Preliminary comparative analyses of the genome of '*Ca.* P. mali' (strain AT) clearly indicate a distinct position and probably a higher level of genome reduction compared to the aster yellows phytoplasmas sequenced so far. This distinct position is also characterized in a different mode of replication, which will be discussed. The comparable small genome size is interpreted as the result of the low amount of duplications and the reduced occurrence of mobile units.

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