Oral Session VIII

First insights into the Genomes of the rich equipped Acholeplasma species highlight the Genome Condensation of the related Phytoplasmas

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Acholeplasmas are thought to be the closest related group of the phytoplasmas. In contrast to the host-depending phytoplasmas Acholeplasma species can be cultivated in established Mollicutes-growth media and several organisms show an ubiquitary distribution.

We determined high-quality draft genome sequences of *Acholeplasma brassicae* str. O502 and Acholeplasma palmae str. J233. These species were selected because of their isolation from plants, their detection in phytoplasma vectors and the phylogenetic proximity to phytoplasmas. The draft genome of A. brassicae, which should be extended to a finished state, contains ca. 1.9 Mb with estimated 1800 protein coding sequences while the genome of *A. palmae* is approx. 1.6 Mb in size with 1500 protein coding sequences. In comparison to *Acholeplasma laidlawii*, whose complete genome sequence is available, both posses a considerable higher amount of coding sequences for transposases/integrases as well as possible paralogs, which all may indicate duplication or insertion events. Besides sequences for DNA-replication and –repair, transcription and translation most identified protein coding sequences are related to the carbohydrate metabolism, the amino acid- and nucleotide-synthesis as well as transport systems for corresponding precursors and products and suggest a nonspecialized and probable opportunistic lifestyle. However, many pathways show analog or differing losses in smaller to larger amount, probably indicating a beginning genome condensation. The content of the protein coding genes of the complete sequenced *Candidatus* Phytoplasma species was compared to *A. laidlawii, A. brassicae* and *A. palmae*. The determined highly interconnected metabolic networks in the *Acholeplasma* species indicate an unexpected distance to the phytoplasma species which is impressive and weakly depicted by the current phylogeny. Typical candidates for virulence associated proteins are missing in both plant-associated groups. Nevertheless, from earlier studies it is indicated that *Acholeplasma* and *Candidatus* Phytoplasma species use comparable insect vectors and therefore probably the same mode of transmission.

Identification of host genes potentially implicated in the *Malus pumila* and *Candidatus* Phytoplasma mali interactions

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Apple proliferation (AP) is one of the most serious diseases of apple trees in Europe. It is caused by a phytoplasma, '*Candidatus* Phytoplasma mali'. The goal of the present study was to analyze transcriptional profiles of *Malus pumila* during infection by 'Ca. P. mali' using cDNA-AFLP technique. A rootstock of apple (MM106) susceptible to 'Ca. P. mali' to maximise the range of the potential host responses, and two strains (AP and AT) of the pathogen were used. Gene expression comparisons were studied in 3 categories of plant materials: healthy sample versus infected samples, symptomatic versus non-symptomatic sample, and AP-infected sample versus AT-infected sample. Forty-five genes whose steady-state levels of expression significantly changed in response to phytoplasma infection were isolated and identified. Of 45 partial cDNA sequences, twenty-seven showed similarity to international DNA or protein data bases. Of these, 18 were previously characterized in plants (the rest was related to unknown or hypothetical proteins). Eighteen out of 45 did not show any similarity with sequences in data bases, and so may be present novel genes. The majority of fragments were differently expressed between healthy sample and infected samples (fewer differences between symptomatic and non-symptomatic samples, or between the samples infected by different strains of phytoplasma). Quantitative Real-time

RT-PCR (qRT-PCR) was used to confirm differential expression of sequences isolated by cDNA-AFLP. Consequently, qRT-PCR showed the similar profile expression as primary elucidation technique (cDNA-AFLP) for 11 known genes (between 18) and 13 unknown, hypothetical or novel genes (between 27). Changes in gene expression involved a wide spectrum of biological functions, including processes of metabolism, cell defence, senescence, photosynthesis, transport, transcription, signal transduction and protein synthesis. The possible effect of phytoplasma infection on these processes and their relationships with disease development, symptom appearance and probably plant defence system is discussed. A model is proposed to explain the mode of action of the 'Ca. P. mali' in its host plant, apple tree.

In vitro screening of interspecific hybrids (*Malus* spp.) for resistance to apple proliferation

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A breeding program was started in 2001 in order to develop apple proliferation (AP) resistant apple rootstocks exploiting the natural resistance found in *Malus sieboldii*.

Twenty-six hybrids generated from the crossings of *Malus sieboldii* and its hybrids with *Malus x domestica* were micropropagated and studied under standardized conditions in vitro. An in vitro screening system for AP resistance previously established for the parental genotypes of the crosses was adopted and further improved. Specific symptoms of the disease i.e. height and basal proliferation of the shoot, and size of the leaves were recorded in vitro at 3 months post inoculation. At the same time phytoplasma concentration was determined in the whole shoot by quantitative PCR. Ex vitro plants were obtained from each culture line and were graft-inoculated *in vivo* in triplicates with two different phytoplasma strains. Phenotype and phytoplasma titre were evaluated in the roots the year after infection.

Preliminary results indicated that the resistance trait segregates in the progenies. The resistant genotypes had lower phytoplasma concentration than the susceptible controls, did not show AP-specific symptoms and their growth was not affected by infection. By comparing the resistant behaviour of the same genotypes, the *in vitro* screening seems to be more severe than the *ex vitro* system thus allowing a quick selection of genotypes that are worth being evaluated in the field for agronomic traits.

Experimental transmission trials by *Cacopsylla pyri,* collected from pear decline infected orchards in Turkey

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A study was carried out on the experimental transmission efficiency of the Pear Decline (PD) phytoplasma by *Cacopsylla pyri* (L.), collected from two naturally infected orchards from Bursa and non-infected orchard from Hatay province of Turkey. *C. pyri* adults were collected for the phytoplasma transmission to healthy periwinkle plants (*Vinca rosea*). Groups of five psyllids per plant were used for transmissison tests and the study was replicated three times. The presence of PD phytoplasma in psyllid transmitted *V. rosea* plants was investigated by using P1/P7 and U3/U5 primer pairs for nested PCR. Although *C. pyri* has limited host range, they were able to survive up to 20 days on periwinkles. Insects collected from the Bursa province survived 16-20 days whereas second group from Hatay, which were fed on PD infected pear for a week, were survived 7-12 days on periwinkles. Symptoms consist of a yellowing or clearing of the veins in newly infected leaves and shortening of the internodes of the main stem. They also remain stunted and flowers were small. All periwinkle plants from the first group showed phytoplasma symptoms whereas only one plant was found symptomatic in the second group. According to the RFLP analysis of Bursa samples, the experimental infection rate of periwinkle plants and psyllids was 33.3% and 16.6%, respectively. No infected periwinkle was found in second group including symptomatic plant but psyllids were 33.3 % infected . Transmission trials under controlled conditions showed the capability of *C. pyri* to transmit PD to healthy test plants and prooved as vector of *Ca. P. pyri* in Turkey.

Analysis of the acquisition and multiplication efficiency of different strains of Ca. Phytoplasma mali by the vector *Cacopsylla picta*

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Based on previous observations during long-term acquisition and transmission trials, studies were carried out under standardized conditions in order to analyse the acquisition and multiplication efficiencies of different strains of *Ca. P. mali* by different developmental stages of *C. picta*. The acquisition of *Ca. P. mali* from micropropagated plants infected with different strains was tested for nymphs, larval stages and new adults of *C. picta*. When born on infected plants a nearly 100% acquisition was achieved for all strains of *Ca. P. mali* by *C. picta*. Differences in acquisition efficiency were observed for new generation adults which acquired the phytoplasma as imagines. The multiplication efficiency of the different *Ca. P. mali* strains inside the insects was analysed by quantitative realtime PCR. Despite high acquisition rates only few subsequent transmission events to healthy test plants could be recorded.

Oral Session IX

Strain differentiation of *Candidatus* Phytoplasma Mali by SSCP- and sequence analyses of the HFLB gene

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Apple proliferation caused by *Candidatus* Phytoplasma mali is an important disease in Central and Southern Europe and reports from Greece and Turkey reflect the spread of the disease. The pathogen itself is known for almost forty years but due to a lacking in vitro culture little biological information is available on the causal agent. Field trials, however, have demonstrated that differences, expressed in virulence, are present. Although, the complete genome sequence of one Ca. P. mali strain is known, the search for genes related to pathogenicity is ongoing. A number of putative candidate genes have been identified, amongst others the *hflB* gene. This gene is present in multiple copies which might reflect its importance for the organism. To explore the *hflB* gene of pathogen strains and isolates in more detail a 530 bp PCR fragment from 42 German, Italian and French accessions were examined. The amplimers were analyzed by singlestrand conformation polymorphism (SSCP) analysis and revealed an unexpected variability. More than 20 different profiles could be discerned. Sequencing of the PCR fragments showed that the nucleic acid homology of the strains ranged from 94.2% to full identity. Although the differences in gene sequence could not yet be related to pathogenicity traits the fragment is predestined as molecular marker for tracking strains in epidemiological studies as well as defining and monitoring inoculum strains in resistance breeding projects.

Molecular characterization of *Candidatus* Phytoplasma mali strains in outbreaks of apple proliferation in north eastern Italy, Hungary, and Serbia

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During 2005-2008 apple plants showing proliferation symptoms were observed in diverse cvs in different areas of north eastern Italy (Veneto and Trentino), Hungary and Serbia. Leaves and young shoots were collected from June to October in orchards with epidemic presence of apple proliferation, and in others where the symptomatic plants were present in a scattered way. PCR/RFLP amplification carried out on R16F2/R2 amplicons showed that all the samples were infected with 'Candidatus Phytoplasma mali'. Further strain characterization was carried out using RFLP analyses with HpaII and FauI on almost full ribosomal DNA plus spacer region, *Alu*I on *rpl22-s3* genes, and RcaI and HincII on AP13/AP10