First report of '*Candidatus Phytoplasma asteris*' associated with napier grass in Cuba

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Napier grass (*Pennisetum purpureum* Schumacher) is one of the most important forage crops in Cuba. In 2013, plant stunting and white leaf were observed affecting over 70% of the surveyed fields in Las Tunas province. Twenty-three leaf samples of plants with symptoms and eight without symptoms were collected. Total DNA was extracted and used in a nested PCR assay to amplify the phytoplasma 16S rDNA gene with primer pairs R16mF1/R16mR1 and R16F2n/R16R2 (Lee et al. 1998). Products of the expected size (1246 bp) were amplified from twenty (86.96%) symptomatic but not from symptomless plants. RFLP patterns of *Pennisetum* white leaf phytoplasma (PWLp) 16S rDNA were divided into two strains, corresponding to variants PWLp-NG49 (six samples) and PWLp-DT165 (14 samples). PWLp-NG49 showed a unique profile using *RsaI* endonuclease. PWLp-DT165 displayed restriction profiles identical to those of reference phytoplasma strains related to '*Candidatus Phytoplasma asteris*' (16SrI-B subgroup, strain AY1) (Lee et al. 1998), using *AluI*, *BstUI*, *HaeIII*, *HhaI*, *HinfI*, *KpnI*, *MseI*, *RsaI* and *TaqI* endonucleases. Three PCR products per strain were cloned and three inserts of each strain were sequenced in both directions (Macrogen, South Korea). Representative sequences

of PWLp-NG49 and PWLp-DT165 were deposited in GenBank (accession Nos. KY000836 and KY000837, respectively). BLASTn search and alignment of related sequences using the MUSCLE algorithm showed that PWLp strains had nucleotide sequence identity of 99.58% with one other, whereas the closest phytoplasma strain *Malvastrum coromandelianum* phyllody (MF490802) showed 99.68% identity with both. PWLp strains displayed 99.52% identity with Maize bushy stunt phytoplasma (strain MBSp-3) (KX925446) and Canavalia yellows phytoplasma (strain CYP 17–25) (KR232799), both detected earlier in Cuba, where '*Ca. P. asteris*' had been previously reported from sugarcane (Arocha et al. 1999). To our knowledge, this is the first evidence of a phytoplasma associated with Napier grass.

References

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