

## Diseases Caused by Bacteria and Phytoplasmas

### First Report of Strains Related to the Phytoplasma Associated with Tanzanian Lethal Decline on *Cocos nucifera* on the Western Coast of Madagascar

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Madagascar is a high-diversity hotspot in the world, and palms are highly represented with nearly 200 endemic species (Rakotoarinivo et al. 2014). Coconut tree (*Cocos nucifera*) could have been introduced in Madagascar by Austronesians around CE 400 or 700 (Beaujard 2011). Sporadic coconut trees showing very severe wilt were observed in 2016 in three localities of the western and northern coast of the island: Katsepy (sample MG16-001), Antsohyhi (MG16-004 and MG16-005), and Ambaritsatrana (MG16-010). Symptoms correspond to a severe ascendant wilt of the leaves, associated with necrosis of the inflorescences and absence of nuts, and death of all trees was confirmed eventually. We investigated the implication of phytoplasma because of the apparent similarity in the symptomatology with coconut lethal yellowing disease and coconut lethal decline occurring in East Africa (Mpunami et al. 1999) and because the western coast of Madagascar faces the Mozambican channel only 400 km apart from areas along the East African coast where those two diseases occur. Symptomatic ( $n = 4$ ) and asymptomatic ( $n = 6$ ) coconut trees were sampled by stem drilling. DNA was extracted from sawdust samples using a modified cetyltrimethylammonium bromide protocol (Mpunami et al. 1999). A direct polymerase chain reaction (PCR) targeting the 16S rRNA gene plus internal transcribed spacer with the P1-1T (AAGAGTTTGATCCTGGCTCAGGAT)/P7 primers (Schneider et al. 1995)

amplified a product of about 1.8 kb for MG16-001 and MG16-005 samples only, whereas the four DNA extracts from symptomatic trees showed a 1.2-kb amplicon by nested PCR using R16F2n/R16R2 primers in the second round (Lee et al. 1998). Amplification of the *secA* gene using the primer pair *secA*For1/sARev3 (Hodgetts et al. 2008) was performed in a single round and gave a product of 850 bp exclusively for the symptomatic tree DNA. All amplicons were double-strand sequenced (Genewiz, U.K.). Corresponding high-quality sequences were deposited in GenBank and submitted to BLASTn on NCBI. The partial 16S rRNA gene sequences (accessions MN264629 to MN264632) obtained using R16F2n/R16R2 primers presented the highest similarity (from 99.47 to 99.56%) to the reference sequence for the phytoplasma associated with the Tanzanian lethal decline (GenBank accession X80117). This genetic proximity of the Malagasy strains was confirmed by the partial *secA* gene sequences (accessions MN267853 to MN267856) presenting the highest similarity (from 89.92 to 90.70%) to the Tanzanian lethal decline phytoplasma *secA* gene partial sequence (GenBank accession KJ462071). Full-length 16S rRNA gene sequences of MG16-001 and MG16-005 strains (accessions MN388765 and MN388766) were submitted to the iPhyClassifier virtual restriction fragment length polymorphism tool (Zhao et al. 2009). The iPhyClassifier tool confirmed that Malagasy strains are related to the reference strain X80117 but belong to a different 16Sr subgroup (similarity coefficient from 0.90 to 0.93, Dev. 1). Both Malagasy strains and Tanzanian lethal decline phytoplasma should be assigned to a new 16Sr group because X80117 is itself erroneously assigned to 16SrIV group, although the closest reference sequence AF509322, 16SrIV-A, shared only a similarity of 0.83 (Dev. 1). Occurrence of a phytoplasma associated with a lethal yellowing-type syndrome in Madagascar could represent a dangerous threat to coconut crops that play an important socio-economic role in the coastal areas, but also to the many endemic palm species already at high risk of extinction.

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