**Phytopathogenic Mollicutes**
Vol. 9 (1), June 2019, 193-194

doi: 10.5958/2249-4677.2019.00097.5



**TROPICSAFE**

# Distribution of lethal yellowing and associated phytoplasma strains in Jamaica, Mexico and other countries in the region

Wayne Myrie[1], Carlos F. Ortíz[2], María Narvaez[3] and Carlos Oropeza[3]

[1]Coconut Industry Board (CIB), Kingston, Jamaica
[2]Colegio de Postgraduados (COLPOS), Tabasco, México
[3]Centro de Investigación Científica de Yucatán (CICY), Mérida, México

## Abstract

The relentless spread of the fatal disease lethal yellowing (LY) throughout the coconut growing areas is having a serious impact on many vulnerable communities. Phytoplasmas from the 16SrIV group are the associated agents. These obligated phytopathogenic mollicutes systemically colonize phloem tissues inducing numerous biochemical and physiological changes leading to symptom development and ultimate death of coconut palms. Large numbers of coconut trees succumbed to the disease over the past three decades. There are different subgroups of the 16SrIV phytoplasmas affecting palms in the Americas. It is important for these to be properly identified along with the epidemiology of the disease. The effective molecular tools used to identify these subgroups show variations in some conserved genomic regions. In the Americas the subgroups have been identified, the current distribution of LY in Jamaica and Mexico and the 16SrIV subgroups occurring in these countries and in other Caribbean countries are described.

**Keywords:** phytoplasmas, coconut lethal yellowing, molecular detection, genetic diversity

## Introduction

The relentless spread of the fatal disease lethal yellowing (LY) throughout the coconut growing areas is having a serious impact on many vulnerable communities. Phytoplasmas from the 16SrIV group are the agents associated with this disease in the Americas and Caribbean areas. These obligated phytopathogenic mollicutes systemically colonize the phloem tissues inducing numerous biochemical and physiological changes leading to ultimate death of coconut palms. There are different subgroups of the 16SrIV groups affecting palms in the Americas. It is important for these to be properly identified along with the epidemiology of the disease.

## Materials and Methods

Samples were collected from coconuts trees displaying LY symptoms and visually healthy trees in Mexico and in some of the islands of the Caribbean area (Figures 1 and 2). DNA extraction was performed from 50 g of trunk boring, using a CTAB extraction protocol (Doyle and Doyle, 1990) PCR amplification was performed on 16S rRNA gene with universal primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) followed by 16SrIV group specific primers 16S503f / LY16Sr (Harrison *et al.*, 1999) in nested PCR assays. Samples were also amplified by groELF1/R1 primers followed by groELF2/R2 (Myrie *et al.*, 2011) in nested-PCR. Positive samples were subjected to RFLP and/or sequencing. The subgroup identification was obtained on 16S rDNA sequences of appropriate size (about 1,200 bp in the 16S ribosomal gene) with the *i*PhyClassifier (Zhao *et al.*, 2009).

**Figure 1.** Symptomatology observed in the coconut trees: yellow leaves in horizontal position and necrotic inflorescences.


**Figure 2.** Distribution of LY disease and some of the places where the samples were collected.

Corresponding author e-mail: Wayne Myrie (waynemyrie@hotmail.com)

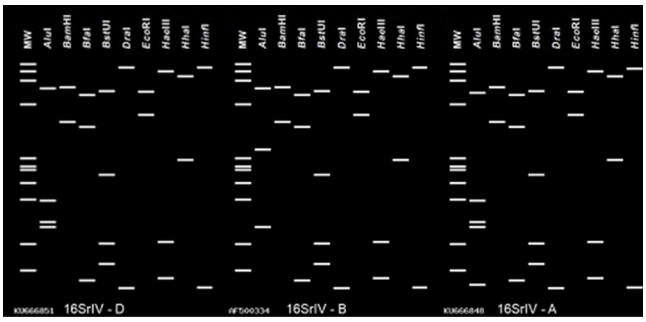


**Figure 3.** Virtual RFLP of amplicons obtained from coconut samples with the enzymes left to right *Alu*I, *Bam*HI, *Bfa*I, *Bst*UI, *Dra*I, *Eco*RI, HaeIII, *Hha*I and *Hinf*I. Amplicon size was 1,249 bp, Ribosomal subgroups are listed at the bottom.

## Results and Discussion

Studies in Jamaica on the diversity of phytoplasmas associated with LY disease revealed that only 16SrIV-A subgroup is affecting the coconut palms. No variations were found in the 16S rRNA and the *groEL* genes in Jamaica. The RFLP characterization on 16S rDNA showed distinct difference with *Alu*I enzyme of the 16SrIV-A and -D strain subgroups (Figure 3). It seems that under favourable conditions all the LY pathotypes can move aggressively through the fields and this can be mitigated by the management strategies which have proven to be effective. Studies on the diversity of phytoplasmas associated to LY or LY-type diseases (LYDs) of palm and non-palm species in Mexico, have shown the occurrence of subgroups 16SrIV-A, -B and –D (Harrison *et al*., 2002). LY disease in Antigua, St. Maarten, St, Kitts and Nevis is associated with the 16SrIV-A subgroup (Myrie *et al*., 2006, 2012, 2014). Subgroup 16SrIV-A is associated with *Cocos nucifera* and other palm species (Cordova *et al*., 2000, 2017) while subgroup 16SrIV-B has been found only in *C. nucifera*. Subgroup 16SrIV-D was identified in *C. nucifera*, other palm species and also the non-palm species *Carludovica palmata* (Vázquez-Euán *et al*., 2011; Narvaez *et al*., 2006, 2016, 2017). Studies have shown that there is a wide geographic distribution of 16SrIV phytoplasmas in the Caribbean *i.e.* Jamaica, St. Maarten/St. Martin, Antigua (Myrie *et al*., 2014) and Barbuda (Myrie *et al*., 2014), St. Kitts and Nevis (Myrie *et al*., 2006, 2012) and in Mexico (Campeche, Yucatan), Gulf of Mexico (Tabasco, Veracruz), Centre (Guanajuato) and South Pacific (Guerrero, Oaxaca).

## Acknowledgements

This work was funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 727459, project "Insect-borne prokaryote-associated diseases in tropical and subtropical perennial crops" TROPICSAFE.

## References

Deng S and Hiruki C 1991. Amplification of 16S rRNA genes from culturable and non culturable Mollicutes. *Journal of Microbiology Methods*, 14: 53-61.

Doyle JJ and Doyle JL 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12(1): 13-15.

Cordova I, Oropeza C, Almeyda H and Harrison NA 2000. First report of a phytoplasma-associated leaf yellowing syndrome of palma jipi plants in southern México. *Plant Disease*, 84: 807.

Cordova I, Mota Narváez L, Puch Hau C, Oropeza C and Sáenz L 2017. Detection and identification of lethal yellowing phytoplasma 16SrIV-A and -D associated with *Adonidia merrillii* palms in Mexico. *Australasian Plant Pathology*, 46: 389-396.

Harrison N, Cordova I, Richardson P and Dibonito R 1999. Detection and diagnosis of lethal yellowing. In: *Current Advances in Coconut Biotechnology*, pp. 183-196. Eds C Oropeza, JL Verdeil, GR Ashburner, R Cardeña and JM Santamaría. Kluwer Academic, The Netherlands

Harrison NA, Narváez M, Almeyda H, Cordova I, Carpio ML and Oropeza C 2002. First report of group 16SrIV phytoplasmas infecting coconut palms with leaf yellowing symptoms on the pacific coast of México. *New Disease Report*, 5: 1-3.

Myrie WA, Paulraj L, Dollet M, Wray D, Been BO and McLaughlin W 2006. First report of lethal yellowing disease of coconut palms caused by phytoplasma on Nevis Island. *Plant Disease*, 90: 834.

Myrie W, Oropeza C, Sàenz L, Harrison N, Roca MM, Còrdova I, Ku S and Douglas L 2011. Reliable improved molecular detection of coconut lethal yellowing phytoplasma and reduction of associated disease through field management strategies. *Bulletin of Insectology*, 64(Supplement): S203-S204.

Myrie WA, Douglas L, Harrison NA, McLaughlin W and James M 2012. First report of lethal yellowing disease associated with subgroup 16SrIV, a phytoplasma on St. Kitts in the Lesser Antilles. *New Disease Reports*, 26: 25.

Myrie WA, Harrison NA, Douglas L, Helmick E, Gore-Francis J, Oropeza C and McLaughlin WA 2014. First report of lethal yellowing disease associated with subgroup 16SrIV-A phytoplasmas in Antigua, West Indies. *New Disease Reports*, 29: 12.

Narvaez M, Cordova I, Orellana R, Harrison NA and Oropeza C 2006. First report of a lethal yellowing phytoplasma in *Thrinax radiate* and *Coccothrinax readii* palms in the Yucatan Peninsula of Mexico. *Plant Pathology*, 55: 292.

Narvaez M, Córdova-Lara I, Reyes-Martínez C, Puch-Hau C, Mota-Narvaez L, Collí A, Caamal G, Harrison N, Sáenz L and Oropeza C 2016. Occurrence of 16SrIV subgroup-A phytoplasmas in *Roystonea regia* and *Acrocomia mexicana* palms with lethal yellowing-like syndromes in Yucatán, Mexico. *Journal of Phytopathology*, 164: 1111–1115.

Narvaez M, Ortíz E, Silverio C, Santamaría J, Espadas F and Oropeza C 2017. Changes observed in *Pritchardia pacifica* palms affected by a lethal yellowing-type disease in Mexico. *African Journal of Biotechnology*, 16: 2331-2340.

Schneider B, Seemüller E, Smart CD and Kirkpatrick BC 1995. Phylogenetic classification of plant pathogenic mycoplasma like organisms or phytoplasmas. In: *Molecular and Diagnostic Procedures in Mycoplasmology*, pp 369-380. Eds S Razin and JG Tully, Academic Press, San Diego, CA, USA.

Vázquez-Euán R, Harrison N, Narvaez M and Oropeza C 2011. Occurrence of a 16SrIV group phytoplasma not previously associated with palm species in Yucatan, Mexico. *Plant Disease*, 95: 256-262.

Zhao Y, Wei W, Lee I-M, Shao J, Suo X and Davis RE 2009. Construction of an interactive online phytoplasma classification tool, *i*PhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *International Journal of Systematic and Evolutionary Microbiology*, 59: 2582-2593.