Phytopathogenic Mollicutes
Vol. 9 (1), June 2019, 195-196

doi: 10.5958/2249-4677.2019.00098.7

TROPICSAFE

Genetic differentiation of the 16SrXXII-B phytoplasmas in Ghana based on the *leucyl tRNA synthetase* gene

Matthew Dickinson[1], Holly Brown[1], Egya Ndede Yankey[2], Sebastian Andoh-Mensah[2] and Felix Bremang[2]

[1]School of Biosciences, University of Nottingham, United Kingdom
[2]Council for Scientific and Industrial Research-Oil Palm Research Institute (CSIR-OPRI), Sekondi, Ghana

Abstract

The Cape St. Paul wilt disease is a lethal yellowing type disease of coconut in Ghana. It is associate with the presence of a phytoplasma belonging to the 16SrXXII-B subgroup also referred to as '*Candidatus* Phytoplasma palmicola'-related strain. A *leucyl tRNA* gene based assay was used to discriminate the two strains of the phytoplasma detected in Ghana since sequences from the 16S rRNA gene were unable to reveal these genetic differences in the phytoplasmas.

Keywords: phytoplasma, CSPWD, Ghana, *leucyl tRNA synthetase* gene

Introduction

The coconut palm is indispensable to the economies of the coastal belt of Ghana. The crop is, however, threatened by a lethal yellowing type disease, locally referred to as Cape St. Paul wilt disease (CSPWD). The disease was first discovered in the South eastern part of the country (Volta Region) in 1932. It subsequently spread to the Western and Central Regions of Ghana in 1964 and 1981 respectively (Ofori and Nkansah-Poku, 1997). CSPWD associated with the presence of phytoplasmas belonging to the 16SrXXII-B subgroup recently officially named '*Candidatus* Phytoplasma palmicola'-related strain (Harrison *et al*., 2014). This subgroup also includes the strain that is associated with the Cote d'Ivoire lethal yellowing disease in the neighbouring Cote d'Ivoire (Arocha *et al*., 2014). While the disease is still active in the Western and Central Regions of Ghana, it has been observed to be less aggressive in the Volta Region (Nkansah-Poku *et al*., 2009). Using a limited number of samples (14 samples from the three coconut growing regions) and based on a ribosomal protein gene, a geographical differentiation of the 16SrXXII-B phytoplasma in Ghana was suggested (Pilet *et al*., 2011). In this study, a ribosomal (16S rRNA) and non-ribosomal (leucyl tRNA) based assays was used to verify the diversity of the 16SrXXII-B phytoplasmas by performing a more extensive sampling across the coconut belt in Ghana.

Materials and Methods

Coconut trunk borings collected from symptomless and CSPWD infected palms in the Western (43); Central (47) and Volta Region (18) of Ghana were the sources of phytoplasma DNA. The samples were collected from palms at all stages of the disease. Three palms in a disease-free area in the Central Region were used as negative controls. DNA extraction was done with a modified Daire *et al.* (1997) protocol using CTAB buffer. Nested PCR targeting the 16S rRNA was carried out using primers P1 (Deng and Hiruki, 1991) and P7 (Smart *et al*., 1996) followed by G813f (Tymon *et al*., 1998) and GAKSR (Dollet *et al*., 2006). The non ribosomal *leucyl tRNA synthethase* gene was amplified using primers described by Abeysinghe *et al.* (2016). All positive PCR samples were sequenced and the sequences compared to NCBI GenBank sequences using the BLAST algorithm. The sequences were aligned and sequence variations investigated using Bioedit version 7. Phylogenetic and molecular evolutionary analyses were performed with MEGA version 7.

Results

Samples from all CSPWD symptomatic palms gave positive results in nested PCR (Table 1). The results from the two assays were consistent with each other. Two samples from symptomless palms also gave positive results. Sequences from the 16S rRNA gene were found to be 100% identical to each other across the sampling locations in the three regions. However, sequencing the *leucyl tRNA synthetase* gene showed that the samples from the Volta Region were distinct from those from the Central and Western Regions with 2 nucleotide differences across the 952 bp length of the sequence (Figure 1).

Corresponding author e-mail: Matthew Dickinson (matthew.dickinson@nottingham.ac.uk)

Table 1. Nested PCR assay of coconut trunk boring samples. DS1-DS4 represents disease stages 1-4; SL: symptomless palm; H: healthy palm. PCR positive/number of samples analysed.

Locations	DS1	DS2	DS3	DS4	SL	H
Western	6/6	13/13	14/14	10/10		
Central	6/6	10/10	13/13	13/13	1/5	0/3
Volta	2/2	3/3	9/9	1/1	1/3	0/3

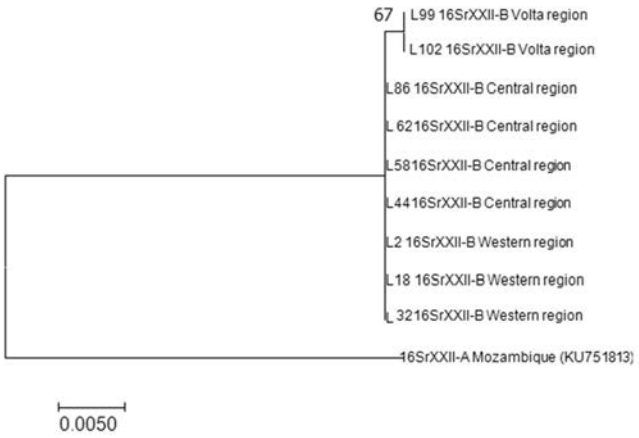

Figure 1. Phylogenetic tree based on the *leucyl tRNA synthetase* gene showing that the phytoplasmas found in the Volta region are distinct from those in the Western and Central Regions (only a few representative samples have been used to draw the tree).

Discussion

The identification of two SNPs in the *leucyl tRNA synthetase* gene of the CSPWD phytoplasma (16SrXXII-B) confirms the geographic differentiation observed by Pilet *et al.* (2011). The distinction between the phytoplasma in the Volta region on one hand, and the Western and Central regions on the other hand may explain the differences in the virulence of the pathogen in the geographic areas as observed by Nkansah-Poku *et al.* (2009). The absence of historical samples or data makes it difficult to determine whether the two strains have evolved independently from each other, or that the original strain that started the disease has mutated or was selected. The identification of two strains of the phytoplasma has implications for resistance breeding experiments: a variety resistant to one strain may succumb to the other. Breeding trials must therefore take this fact into account.

Acknowlegements

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

Abeysinghe S, Abeysinghe PD, Kanatiwela-de S, Chamin U, Preethi W, Kanjana N, Kawicha P and Dickinson M. 2016. Refinement of the taxonomic structure of 16SrXI and 16SrXIV phytoplasmas of gramineous plants using multilocus sequence typing. *Plant Disease*, 100: 2001-2010.

Arocha-Rosete Y, Konan Konan JL, Diallo AH, Allou K and Scott JA 2014. Identification and molecular characterization of the phytoplasma associated with a lethal yellowing-type disease of coconut in Côte d'Ivoire. *Canadian Journal of Plant Pathology*, 36: 141–150.

Daire X, Claire D, Reinert W and Boudon-Padieu E 1997. Detection and differentiation of grapevine yellows phytoplasmas belonging to the elm yellows group and to the "stolbur" subgroup by PCR amplification of non-ribosomal DNA. *European Journal of Plant Pathology*, 103: 507-514.

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

Dollet M, Fabre S, Pilet F, Quaicoe R, Mugini JA and Rassaby L 2006. Variability and diagnosis of coconut lethal yellowing syndromes in Africa. *16th International Congress of the International Organization for Mycoplasmology (IOM)*, Cambridge, UK.

Harrison N, Davis RE, Oropeza C, Helmick E, Narvaez M, Eden-Green S, Dollet M and Dickinson M 2014. '*Candidatus* Phytoplasma palmicola', a novel taxon associated with a lethal yellowing-type disease (LYD) of coconut (*Cocos nucifera* L.) in Mozambique. *International Journal of Systematic and Evolutionary Microbiology* 64: 1890–1899.

Nkansah-Poku J, Philippe R, Quaicoe RN, Dery SK and Arthur R 2009. Cape Saint Paul wilt disease of coconut in Ghana: surveillance and management of disease spread. *Oleagineux*, 16: 111-115.

Ofori F and Nkansah-Poku J 1997. Cape Saint Paul wilt disease of coconut in Ghana: history of its occurrence and spread. In: *Proceedings of an International workshop on lethal yellowing-like diseases of coconut*, pp 27-32. Eds SJ Eden-Green and F Ofori. Chatam, UK: Natural Resources Institute.

Pilet F, Poulini L, Nkansah-Poku J and Quaicoe R 2011. Ribosomal protein gene sequences reveal a geographical differentiation between CSPWD phytoplasmas in Ghana. *Bulletin of Insectology* 64(Supplement): S219–S220.

Smart C, Schneider B, Blomquist CL, Guerra LJ, Harrison NA, Ahrens U, Lorenz KH, Seemüller E and Kirkpatrick BC 1996. Phytoplasma-specific PCR primers based on sequences of the 16–23S rRNA spacer region. *Applied and Environmental Microbiology*, 62: 2988-2993.

Tymon AM, Jones P and Harrison NA 1998. Phylogenetic relationships of coconut phytoplasmas and the development of specific oligonucleotide PCR primers. *Annals of Applied Biology*, 132: 437-452.