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Ribosomal protein gene sequences reveal a geographical differentiation between CSPWD phytoplasmas in Ghana

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Abstract

In Ghana, coconut lethal yellowing phytoplasma locally called Cape Saint Paul Wilt Disease (CSPWD) is the most damaging coconut disease. Two different *foci* of the disease can be distinguished: the first one covering the coast of the Western and Central Regions, and the second covering the coast of the Volta region. To test the hypothesis of a genetic differentiation between the CSPWD phytoplasma from the two *foci*, the partial ribosomal operon 16S, and two ribosomal protein genes (*rplV* and *rpsC*) of 14 strains were sequenced. The ribosomal protein gene sequences allowed the differentiation of the strains originating from the two different *foci* by a unique SNP, confirming a genetic differentiation.

Key words: CSPWD, phytoplasma, lethal yellowing disease, coconut, ribosomal protein genes, genetic diversity.

Introduction

In West Africa, coconut lethal yellowing is the most damaging coconut disease. It has been reported in Ghana, Nigeria and Togo (Dery *et al.*, 1997), and it is associated with a phytoplasma of the 16SrXXII group (Wei Wei *et al.*, 2007).

In Ghana, the disease is locally called Cape Saint Paul Wilt Disease (CSPWD) because it was first observed in 1932 at Cape Saint-Paul, in the eastern Volta region.

While the disease was still active in Togo and Volta region, it was further detect 350 km west at Cape Three Points in Western Region in 1964. In Central Region the disease was reported at Ayensudu in 1983. The disease was spreading and is still active in the different regions since first disease reports.

However, a disease-free gap of about 200 km between the Western-Central Regions and the Volta Region can be observed.

Because of both the history of the CSPWD and its geographical distribution in Ghana, we tested the hypothesis of a regional differentiation by sequencing the 16S rDNA gene and the 16S-23S rDNA intergenic region, as well as two ribosomal protein genes *rplV* (*rpl22*) and *rpsC (rps3)*, which have been described as permitting a finer differentiation of phytoplasma strains (Martini *et al.*, 2007), for isolates sampled in the different regions.

Materials and methods

Two ribosomal protein genes (*rplV* and *rpsC*) were sequenced from 14 CSPWD coconuts trees strains collected in Volta (6), Central (3) and Western (5) Regions in 2006 (2), 2008 (1) and 2009 (11) (figure 1, table 1). The partial ribosomal RNA operon was sequenced only for six of those isolates collected in 2009 in Volta (2), Central (2) and Western (2) Regions.

DNA extraction of all the CSPWD samples was performed using a CTAB protocol. The partial ribosomal RNA operon was amplified using the phytoplasma universal primers P1 (Deng and Hiruki, 1991) and P7 (Smart *et al.*, 1996). The *rp* gene operon was amplified using the PCR primer pair rpLYF1 (5'-TTT AAA GAA GGT ATT ACA TGA-3') and rpLYR1 (5'-TAA TAC CTA TAA CTC CGT G-3') designed by Marinho *et al.*, (2006). PCR conditions were 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 50°C for 1 minute and 72°C for 2 minutes and a final extension step of 72°C for 7 minutes.

The P1/P7 PCR products were directly sequenced. Products of the rpLYF1/rpLYR1 PCR showing a clear and strong amplification were directly sequenced while PCR products showing a weak amplification were cloned into the pGEM-T Vector (Promega) and purified using QIAprep Miniprep (Qiagen) according the manufacturer's instructions before sequencing. The sequencing was done by Beckman Coulter Genomics. The sequences were aligned by using Clustal W and analyzed by using DnaDist under Bioedit version 7.0.9.0 program software.

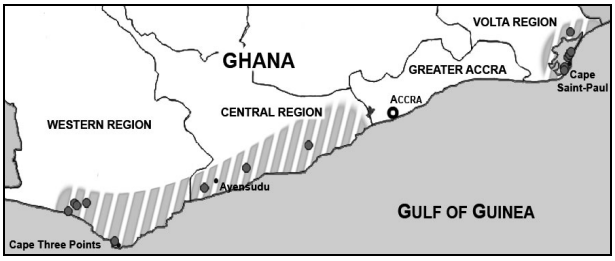

Figure 1. Geographical distribution of the 14 samples analyzed in this study and schematization of the two distinct CSPWD foci in Ghana.

Table 1. Sequences *rplV-rpsC* from nucleotide positions 340 to 351 for the 14 samples presented in this study (*sample sequenced also for P1/P7), their region of origin (VR: Volta Region, CR: Central Region, WR: Western Region) and the sampling year.

Sample Name	Region	Sampling Year	*rp* sequence (340-351)
GH06-TET	VR	2006	ACG TCA **C**AT AAT
GH06-WOE	VR	2006	... ... **C**.. ...
GH09-001*	VR	2009	... ... **C**.. ...
GH09-006	VR	2009	... ... **C**.. ...
GH09-015*	VR	2009	... ... **C**.. ...
GH09-022	VR	2009	... ... **C**.. ...
GH09-051	CR	2009	ACG TCA **A**AT AAT
GH09-069*	CR	2009	... ... **A**.. ...
GH09-090*	CR	2009	... ... **A**.. ...
GH08-127	WR	2008	... ... **A**.. ...
GH09-102	WR	2009	... ... **A**.. ...
GH09-111*	WR	2009	... ... **A**.. ...
GH09-121*	WR	2009	... ... **A**.. ...
GH09-125	WR	2009	... ... **A**.. ...

Results

The primer pair P1/P7 amplified a 1,756 bp fragment. The six P1/P7 amplified sequences from the three different geographic regions were 100% identical to each other.

Amplification of the two ribosomal protein genes *rplV* and *rpsC* using rpLYF1/rpLYR1 produced a band of 983 bp. A total of seven mutations were observed between the sequences of the rpLYF1/rpLYR1 region with a similarity between 99.53 and 100%. Six of the mutations were present in only one strain. The last mutation, at nucleotide position 346, was common to all the strains from the same region of origin.

At this position, an adenosine (A) is present for strains originating from Western and Central regions, while a cytosine (C) can be observed for the strains from Volta region, independent of the year they were collected (table 1).

Discussion

Sequencing of the ribosomal protein genes *rplV-rpsC* reveals a geographical differentiation of the CSPWD phytoplasma in Ghana by a unique SNP at nucleotide position 346, whereas the partial ribosomal RNA operon failed to detect genetic variability. This geographical differentiation suggests an independent evolution of the two CSPWD *foc*i without exchange of phytoplasma strains between them.

However, the number of samples sequenced in this study is small and analysis of a larger number of strains using the restriction enzyme *Tsp*45I, which is able to recognize the SNP will be necessary to confirm the results.

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