



**HAL**  
open science

## Deletion of the cassiicolin-encoding gene Cas1 from *Corynespora cassicola* causes a loss of virulence on rubber tree

Sébastien Ribeiro, Dinh Minh Tran, Marine Deon, André Clément-Demange, Dominique Garcia, Mouman Soumahoro, Aurélien Masson, Valérie Pujade-Renaud

### ► To cite this version:

Sébastien Ribeiro, Dinh Minh Tran, Marine Deon, André Clément-Demange, Dominique Garcia, et al.. Deletion of the cassiicolin-encoding gene Cas1 from *Corynespora cassicola* causes a loss of virulence on rubber tree. 12th Congress of the International Plant Molecular Biology, IPMB 2018., Aug 2018, Montpellier, France. 1 p. hal-02089525

**HAL Id: hal-02089525**

**<https://hal.science/hal-02089525>**

Submitted on 4 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Deletion of the cassiicolin-encoding gene *Cas1* from *Corynespora cassiicola* causes a loss of virulence on rubber tree

Sébastien Ribeiro<sup>1,2,3</sup>, Dinh Minh Tran<sup>2,3,4</sup>, Marine Déon<sup>1</sup>, André Clément-Demange<sup>2,3</sup>, Dominique Garcia<sup>2,3</sup>, Mouman Soumahoro<sup>5</sup>, Aurélien Masson<sup>6</sup>, Valérie Pujade-Renaud<sup>1,2,3</sup>



<sup>1</sup>Université Clermont Auvergne, Institut National de la Recherche Agronomique, UMR PIAF, Clermont-Ferrand, France

<sup>2</sup>AGAP, Université Montpellier, CIRAD, Institut National de la Recherche Agronomique, Montpellier, France

<sup>3</sup>CIRAD, UMR AGAP, F-63000 Clermont-Ferrand, France

<sup>4</sup>Rubber Research Institute of Vietnam, Ho Chi Minh City, Vietnam

<sup>5</sup>Société Africaine de Plantations d'Hévéa, 01 BP 1322 Abidjan 01, Côte d'Ivoire

<sup>6</sup>Société des Caoutchoucs de Grand-Béréby, Grand Béréby, Côte d'Ivoire



## Background

Rubber tree (*Hevea brasiliensis*) is an economically important tropical tree that provides natural rubber. In Asia and Africa, it is affected by the Corynespora Leaf Fall (CLF) disease caused by the necrotrophic fungus *Corynespora cassiicola*. On the most susceptible rubber clones, *C. cassiicola* can cause massive defoliation leading to yield losses. Some pathogenic strains secrete a small protein toxin, the cassiicolin<sup>1,2,3</sup>, which is transcriptionally up-regulated during early stages of the disease<sup>4</sup>. Seven isoforms were identified and used to classify the various strains into toxin classes<sup>5</sup>. Strains belonging to toxin class **Cas1** were the most aggressive on tested rubber clones<sup>5</sup>. However, strains without cassiicolin gene (toxin class **Cas0**) may also produce toxic exudates<sup>6</sup>, suggesting the existence of effectors other than cassiicolin.



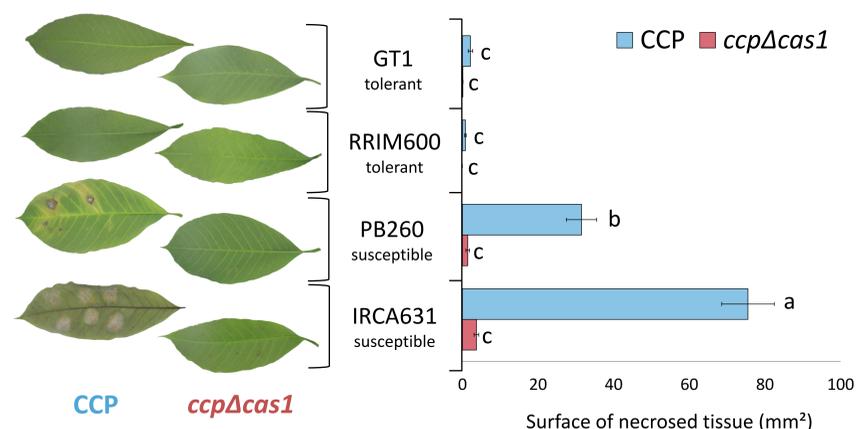
susceptible clone



tolerant clone

## Virulence of the *ccpΔcas1* strain

CCP and *ccpΔcas1* strains were compared for their virulence on four rubber clones (still attached to the plant), by analyzing the extent of symptoms as the mean surface of necrotic tissue (mm<sup>2</sup>), nine days after inoculation (200 conidia/drop, 6 drops per leaf).



**Result 2:** Without cassiicolin Cas1, the CCP strain is avirulent on susceptible clones, except for a few pinpoint symptoms.

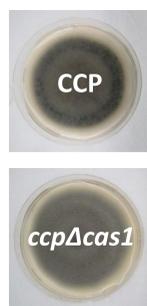
## Objective

To determine the importance of cassiicolin *Cas1* for the virulence of *C. cassiicola* strain CCP, comparatively to others putative effectors, by comparing the wild-type strain and the same strain deleted for the cassiicolin-encoding gene *Cas1*, in interaction with rubber tree.

## Construction of the *Cas1* gene deletion mutant

Deletion mutant was obtained by replacing the *Cas1* gene from the highly aggressive strain CCP with a hygromycin-resistance cassette by homologous recombination.

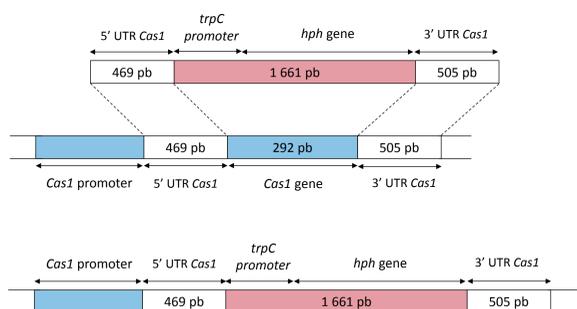
The deletion mutant was named *ccpΔcas1*.



hygromycin cassette

wild-type CCP genome

deletion mutant *ccpΔcas1* genome



## QTL associated with sensitivity of rubber tree to cassiicolin *Cas1*

QTL detection was conducted on the PB260 x RRIM600 family for the response to purified cassiicolin *Cas1*, to culture of isolates carrying or not a *Cas1* gene (including CCP and *ccpΔcas1*) and to blank treatments (culture medium and water). The response was quantified by conductivity measurement of the induced electrolyte leakage<sup>6</sup>.

Treatment	g2-26	g3-11	g4-95	g4-32	g5-73	g6-26	g9-62	g9-122	g12-53	g13-102	g14-64	g16-11	g16-77	g18-87
Purified cassiicolin <i>Cas1</i>	17	-	17	-	-	-	-	-	-	-	-	-	-	-
CCP - <i>Cas1</i>	12	-	13	-	14	-	-	-	-	-	-	-	-	-
<i>ccpΔcas1</i> - <i>Cas0</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCi501 - <i>Cas1</i>	16	-	19	-	-	-	-	-	-	-	-	-	-	-
CNi404 - <i>Cas1</i>	20	-	12	-	-	-	-	-	-	-	-	-	-	-
CCi434 - <i>Cas0</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCi403 - <i>Cas0</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIND3 - <i>Cas0</i>	-	-	-	-	-	-	-	11	-	-	-	-	11	-
CLN16 - <i>Cas0</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CTHA3 - <i>Cas0</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culture medium (blank)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Water (blank)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Toxin class (*Cas1* or *Cas0*) is indicated for each isolate.

QTL are named by the number of the linkage group (g) on which they are located, and their position. Numbers represent the percentage of explained phenotypic variance.

**Result 3:** At least two QTL (in yellow) were detected with the purified cassiicolin and with *Cas1* culture filtrates, but none with *ccpΔcas1*.

## Physiological analyses of CCP and *ccpΔcas1*

The growth rate, conidia production and percentage of germination were compared *in vitro* between CCP and *ccpΔcas1* strains.

Strain	Growth rate (mm/day)	Conidiation (conidia/μl)	Germination (%)
CCP	4.13 ± 0.16a	413 ± 77a	71.75 ± 9a
<i>ccpΔcas1</i>	4.29 ± 0.08a	479.5 ± 42a	69.75 ± 6a

**Result 1:** Deletion of the *Cas1* gene did not modify major physiological functions in the CCP strain.

## Conclusion

Cassiicolin *Cas1* is the major virulence factor involved in the compatible interaction between CCP and susceptible rubber clones. It could be a good candidate for effector-based selection.

## REFERENCES

- BRETON, F. (2000) Journal of Natural Rubber Research 3, 115–128.
- BARTHE, P. (2007) Journal of Molecular Biology 367, 89–1013.
- DE LAMOTTE, F. (2007) Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 849, 357–362.
- DÉON, M. (2012a) Plant science : an international journal of experimental plant biology 185–186, 227–237.
- DÉON, M. (2014) Fungal Biology 118, 32–47.
- TRAN, D.M. (2016) PLOS ONE 11, e0162807. Public Library of Science.