

Introdução/Introduction

Cryphonectria parasitica, the causal agent of chestnut blight, causes necrotic lesions (so-called cankers) on the bark of stems and branches of susceptible host trees. *Cryphonectria hypovirus* 1 (CHV1) infects *C. parasitica* reducing its parasitic growth, sporulation capacity, female fertility, pigmentation and laccase production (Rigling & Prospero, 2018). Due to its effects CHV1 is used in Europe as a biological control agent of the chestnut blight fungus by hypovirulence.

Objetivos/Objectives

The aim of this project was to understand the effect of CHV1 presence on *C. parasitica* behaviour by comparing the production of some lignin degrading enzymes in virulent and hypovirulent (naturally hypovirulent and hypovirulent by conversion) strains, as well as its metabolic profiles.

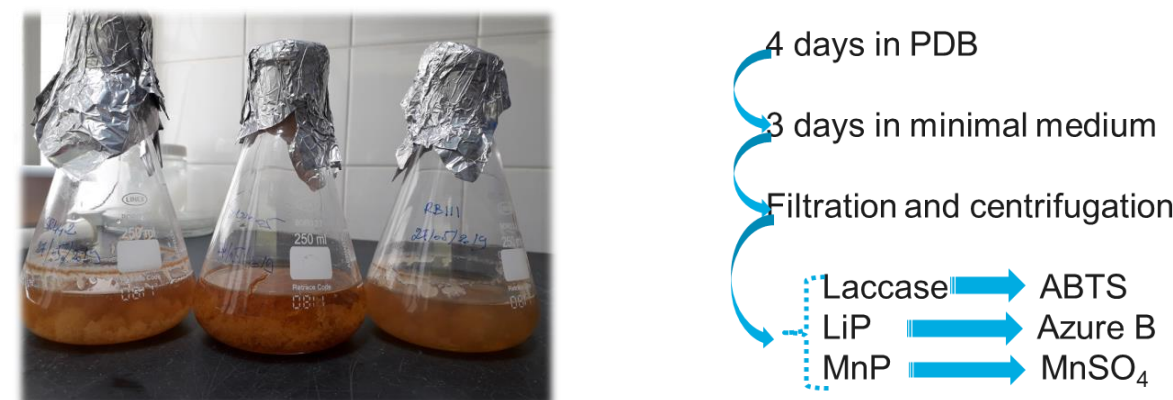
Material e Métodos /Material and Methods

C. parasitica isolates used in this work were: the virulent Vbc02 and Cast13, the converted Vbc02c and Cast13c, and the naturally hypovirulent RB111 - wild (used for Vbc02 and Cast13 conversion).

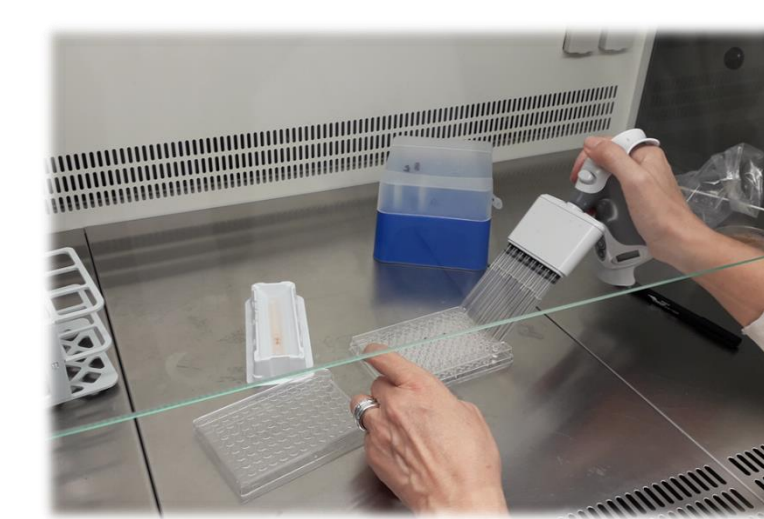
1. The degree of virulence of *C. parasitica* strains (virulent and hypovirulent) was evaluated either by inoculation in apples and in young branches of *Castanea sativa*.



2. The production of acid tannic inducible laccase (lac3), lignin and manganese peroxidases (LiP and MnP) in virulent and hypovirulent strains of *C. parasitica* were determined.



3. The differences in metabolic profile of *C. parasitica* virulent and hypovirulent strains were assessed

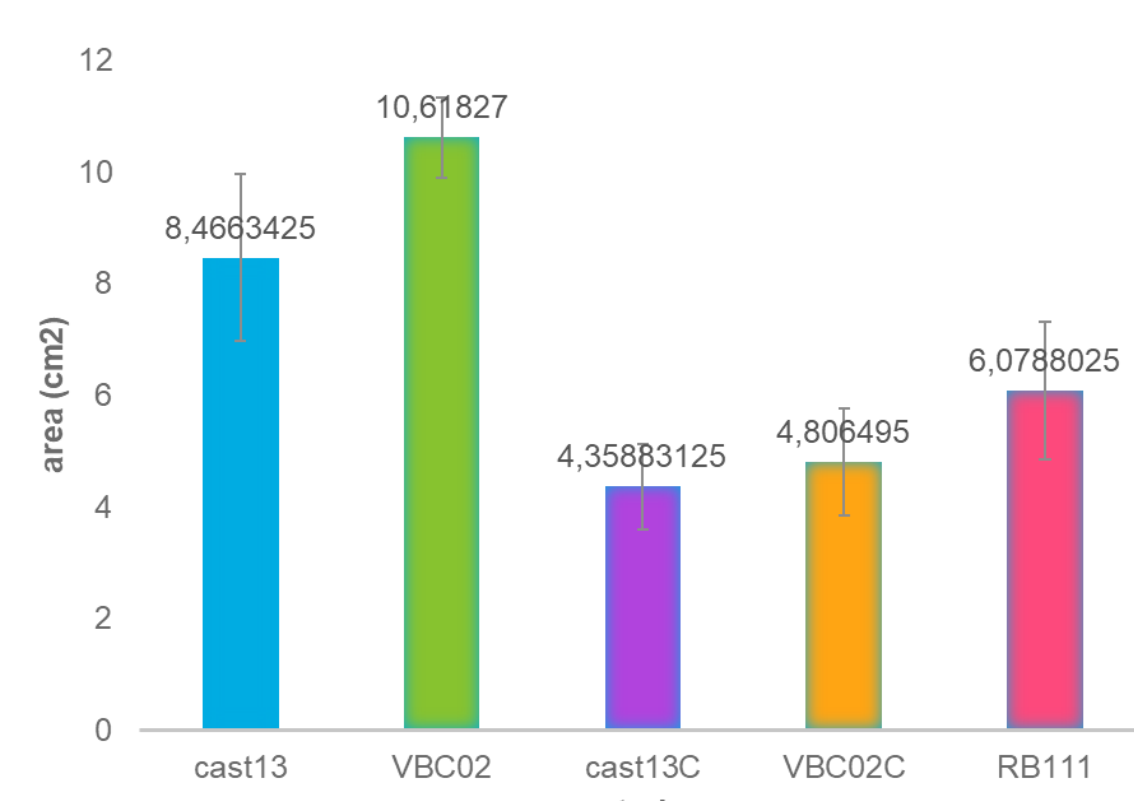


- A plate with 95 different low molecular weight carbon sources
- inoculated with a fluid suspended with smashed mycelial
- OD at 490nm determined with a microplate reader

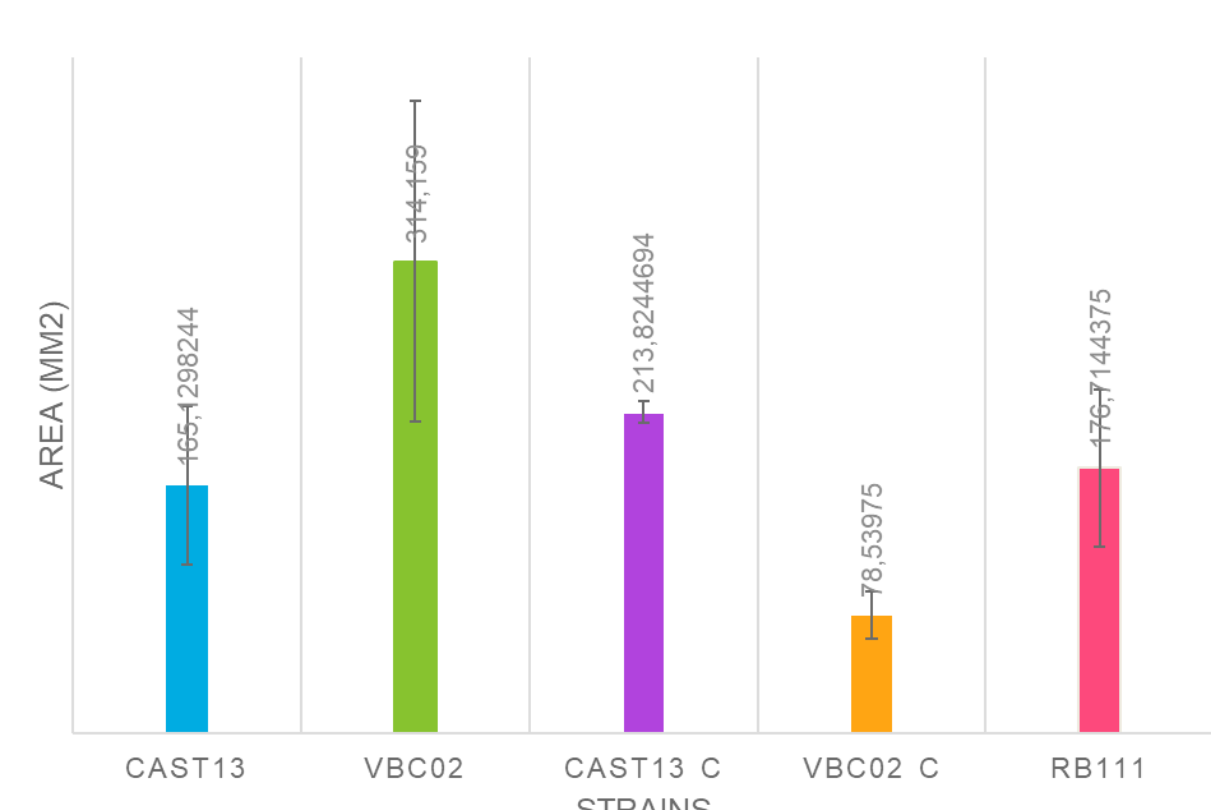
Resultados/Results

1. Virulence tests

Lesions in chestnut branches

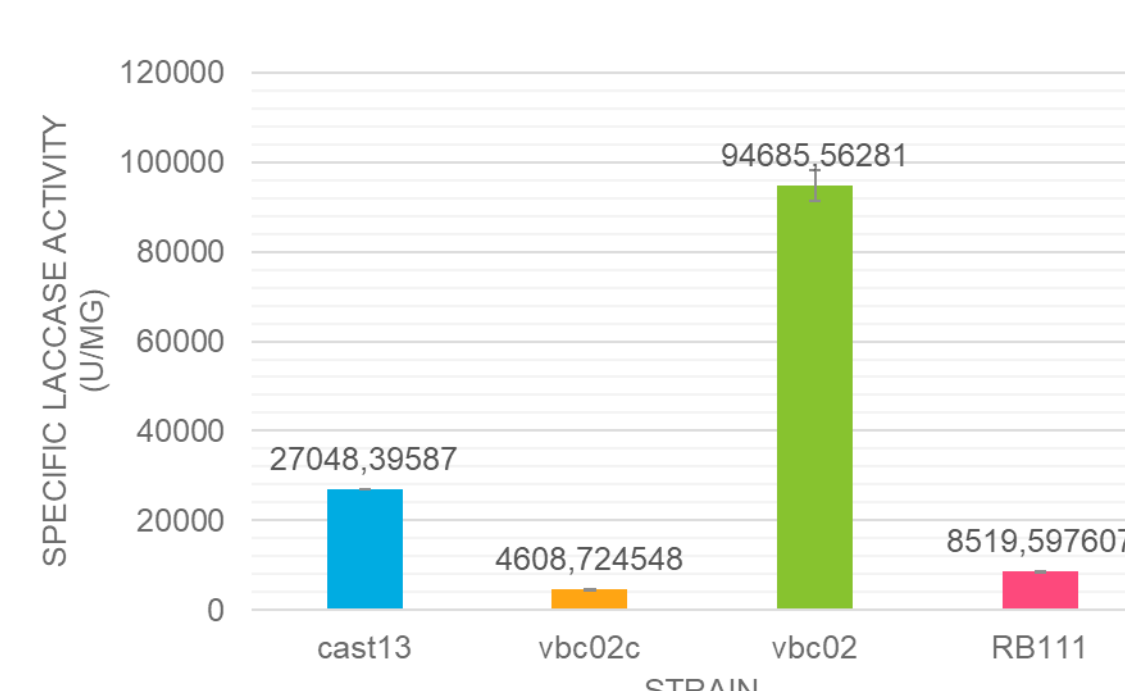


Rot lesions in apple fruits

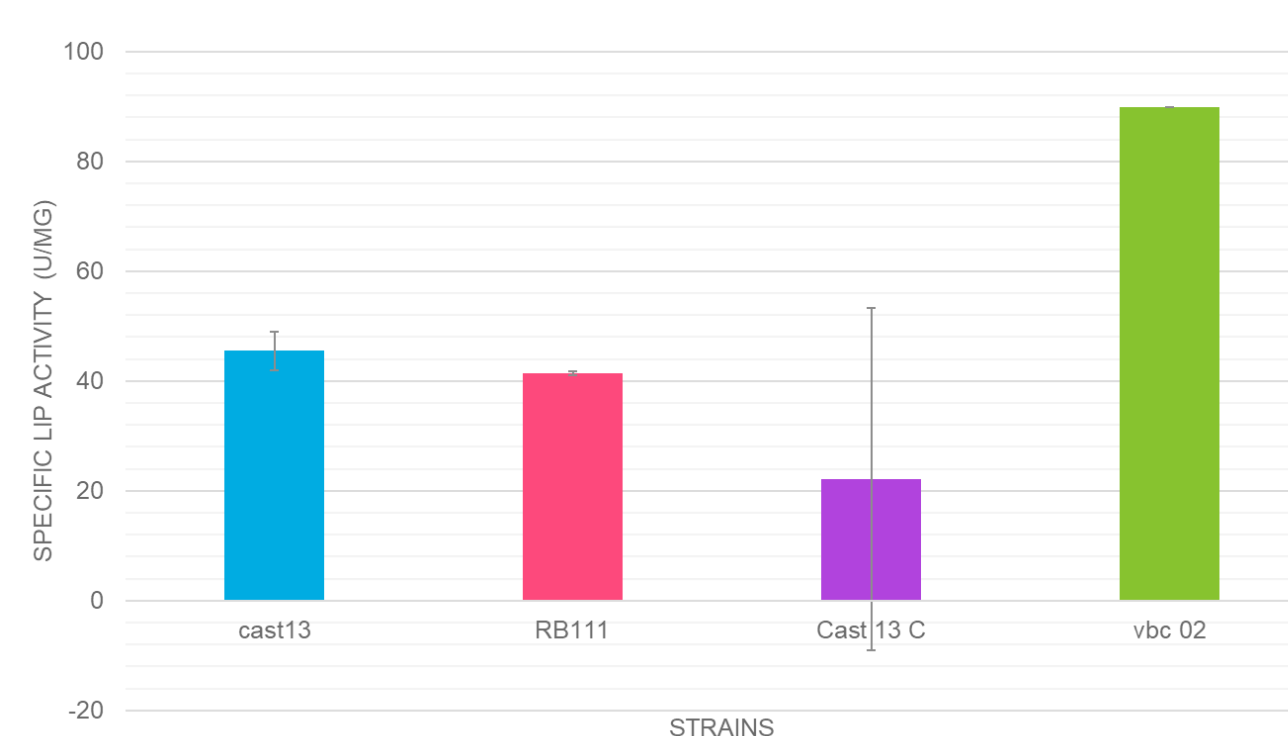


2. Ligninolytic enzyme production

Laccase activity

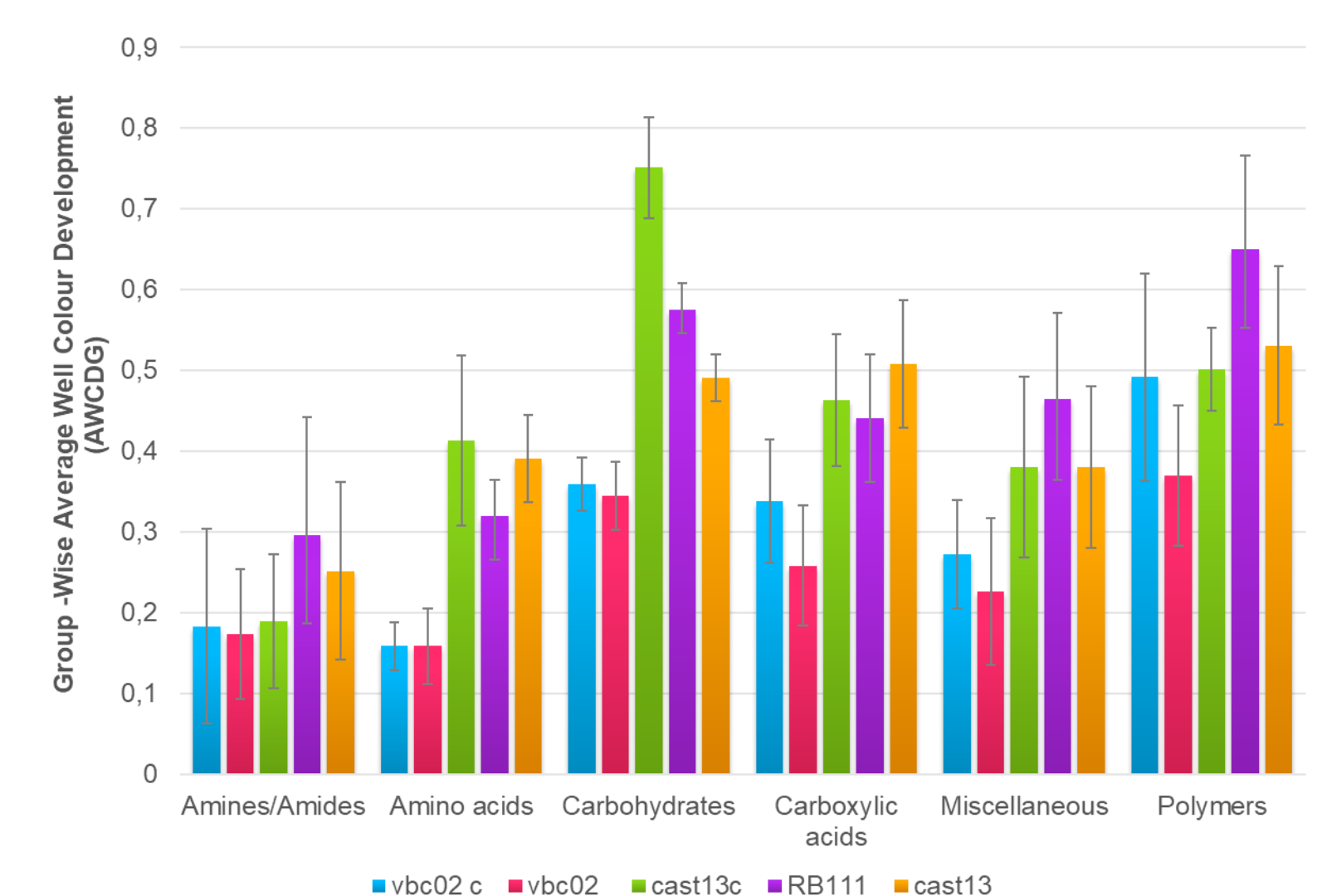


Lignin peroxidase activity



- no MnP activity detected

3. BIOLOG FF Microplates results



- The isolate Vbc02c revealed a higher capacity in the utilization of metabolites when compared with Vbc02.
- On the other hand, Cast13c showed higher utilization of carbohydrates and amino acids than Cast13.
- RB111 displayed higher utilization of metabolites when compared with the virulent isolates, with exception of the group of the carboxylic acids.
- When compared with the converted isolates, RB111 showed higher metabolite utilization only in the group of the amines/amides, miscellaneous compounds and polymers.

Conclusões/Conclusions

This work shows the enormous changes that happens in response to the hypovirus, proving that the mycovirus somehow keeps the mycelium in juvenile state with the induction of alternative metabolic pathways.

New study approaches could be explored such as :

- the differences between an wild hypovirulent strain and a newly infected strain (converted)
- the process behind this adaptation mechanism in the fungus, that happens after the introduction of the virus
- a new database for *Cryphonectria parasitica* could be created using BIOLOG microplates for metabolic profiles characterization

Referências/References

Rigling, D. and Prospero S. 2018., “*Cryphonectria Parasitica*, the Causal Agent of Chestnut Blight: Invasion History, Population Biology and Disease Control.” *Mol. Plant Pathol.* 19(1):7-20. doi: 10.1111/mpp.12542. Epub 2017 Apr 24.

Agradecimentos/Acknowledgments

Work supported by the project PDR2020-101-030943 - “BioChestnut- IPM - Implementar estratégias de luta eficazes contra doenças do castanheiro e amendoeira”.

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2019).