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# Purification and structure analysis of Cassiicolin, primary determinant of *Corynespora cassiicola* pathogenesis on rubber tree

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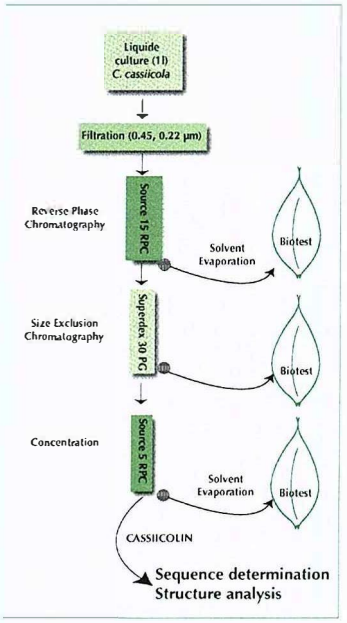
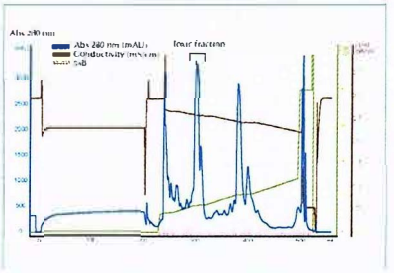
*During the past decade, the necrotrophic fungus* Corynespora cassiicola *has caused extensive damage to rubber tree plantations in all producing areas through massive defoliation.*
*Filtrates from* C. cassiicola *cultures contain a phytotoxic substance (cassiicolin) able to induce the disease symptoms on leaves. This toxin was shown to be an essential determinant of* C. cassiicola *pathogenicity on rubber tree (Breton* et al.*, 2000).*
*An optimized protocol for the purification of cassiicolin is presented, together with the first representation of Cassiicolin 3D structure.*

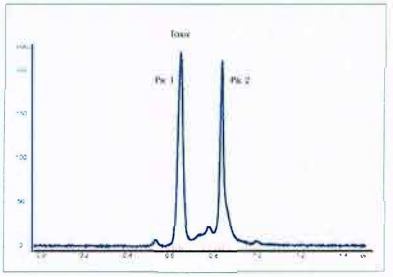
Cassiicolin purification protocol.

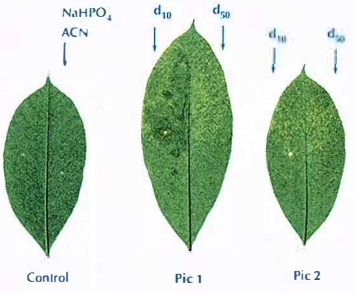


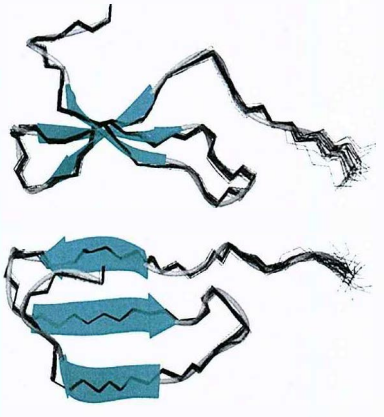

Fractionation on a Source 15RPC column (Amersham Biosciences) and toxicity monitoring on rubber tree detached leaves (biotest).




Fractionation on Superdex 30 PG (Amersham Biosciences) and toxicity monitoring on rubber tree detached leaves (biotest).





3D structure of cassiicolin as determined by NMR. Superposition of 30 NMR structures of Cassiicolin over the backbone heavy atoms (in black) and the ribbon diagram of the mean structure showing the secondary structure elements. The two views are related by a 90° rotation around the horizontal axis.

The final purified fraction is composed of a major product of 2884.8 Da and minor product of 2708.9 Da which may correspond to the loss of a 176 Da sugar moiety. This moiety was also easily detached from the major product by MS-MS.

## Conclusion

An optimized protocol was set up for the purification of cassiicolin, toxin responsible for the pathogenicity of *Corynespora cassiicola* isolates from rubber tree. Cassiicolin is a 27 amino acids glycoprotein, with a molecular mass of 2885 Da. No significant homology was detected by amino acid sequence blast analysis against public databases. Cassiicolin 3D structure was determined by NMR. Comparison of toxins from *C. cassiicola* strains of different geographical origins is ongoing, to assess whether variability in the toxin structure may be responsible for differences in pathogenicity.

## References

Breton F., Sanier C., d'Auzac J. (2000). Role of cassiicolin, a host-selective toxin, in pathogenicity of *Corynespora cassiicola*, causal agent of a leaf fall disease of Hevea.

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CIRAD French Agricultural Research Centre for International Development
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