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3D NMR structure of a new Cyt-like δ-endotoxin from *Dickeya dadantii*

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Introduction: Among insects, aphids (Hemiptera: Aphidoidea) are one of the most injuring taxa for agricultural plants. Until now, most aphid pest control strategies rely on the use of specific sets of systemic chemical pesticides. But the extensive use of these pesticides had led to resistance to insecticides in several aphid species, and cause significant environmental damage by targeting different guilds of beneficial insects (predators, parasitoids, and pollinators). Therefore, it is highly desirable to develop biopesticides with low off-target effects. Plant bioengineering has also led to some field insect resistance, and very few genetically modified plants have yet been developed with resistance to sap-sucking insects (none is used commercially).



In the track of new biopesticides, several genes encoding proteins homologous to *Bacillus thuringiensis (Bt)* Cyt toxins have been identified in the plant pathogenic bacteria *Dickeya dadantii* genome. Three Cyt-like δ -endotoxins from *D. dadantii* (namely *cytA*, *cytB*, *cytC*) are toxic to the pathogen of the pea aphid *Acyrthosiphon pisum* in terms of both mortality and growth rate.

To better understand the role of *D. dadantii* Cyt toxins in its pathogenicity to insect, we defined the following strategy for the present study:

- i) the four recombinant proteins were purified and were used for toxicity bioassays against the pea aphid *A. pisum*;
- ii) a phylogenetic analysis was performed to investigate the evolutionary and functional relationships within the whole Cyt-like protein family,

iii) from a structure-function perspective, the CytC 3D structure and its dynamics in solution have been determined by NMR.

CytC is structurally a Cyt2-like protein

Sample : 0.3 mM ¹⁵N, ¹³C labelled CytC protein in 90% H₂O, 10% D₂O, 50 mM Tris (pH 7.0), 100 mM NaCl, 1 mM EDTA , 5 mM DTT, 298K

NMR: 600MHz, ¹⁵N-HSQC-NOESY / ¹³C-HSQC-NOESY / ¹⁵N R₁ and R₂/ ¹⁵N-NOE spectra. 95% of the backbone and 75% of the proton side-chains assigned



The hydrophobic residues delineating the cavity are conserved among the Cyt family members → the common ancestor of Evf and the cytolytic toxins contained a lipid binding

site which has been maintained in the two

clades, and probably in most members of the

NTADESKNESVTLNV

PSSDLDNFNTVFYVO

SYALOAEODOSLHFKTLFSVD

Cyt-toxin family.

daCytC|1-20

DdaCytA|11-21 DdaCytB|2-197

DdaCvtDl26-21

Cyt1Aa(3RON)

yt1Ab|25-23

vt1Ba|38-24

Cyt1Ca|19-226 Cyt2Aa(1CBY)|29-;

Cyt2Bc|27-22

DdaCytC|1-20

DdaCytA|11-210 DdaCytB|2-197

DdaCytD|26-218 Cyt1Aa(3RON)|25

Cyt1Ab|25-232

Cyt1Ba|38-244 Cyt1Ca|19-226

Cyt2Aa(1CBY)|29-Cyt2Ba(2RCI)|30-Cyt2Bb|27-225 Cyt2Bc|27-225 Cyt2Ca

DdaCytC|1-203 DdaCytA|11-210 DdaCytB|2-197

DdaCytD|26-21 Cyt1Aa(3RON)|2

Cyt1Ba|38-244 Cyt1Ca|19-226 Cyt2Aa(1CBY)|29-

vt2Bcl27-22

Cyt2Ba(2RCI)|30-2

Contières

CBW

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Front to back : BRUKER AVANCEIIIHD 700MHz with TCI cryoprobe, BRUKER AVANCEIIIHD 400MHz, BRUKER AVANCEIII 600MHz



Dda-Cyt genes encode insecticidal proteins active against the pea aphid, and this activity is at least as potent as that of its parent Bth-Cyt1A protein.



(BMRB code 19834).

Softwares: NMRPIPE/ CCPNMR/ARIA2/ TALOS+ /DANGLE

Coordinates : PDB entry 2MLW (20 models 2 conformations)





CytC display a cytolysin fold, i.e. an anti-parallel 4-stranded β-sheet having a modified Greek key topology, flanked by two α-helical layers.

Two distinct and equally populated conformations of the protein in solution, in a fast exchange regime. The "closed" conformation (models 1 to 10 - left side), in which $\alpha 4$ is closer to the β -sheet, exhibits a **quite large hydrophobic pocket** (~1000Å³), whereas this pocket is accessible in the "opened" conformation (models 11 to 20 – right side)

The structural homology between Cyt proteins and Evf (despite its very low (~15%) sequence identity) enabled the identification of a putative fatty acid binding site in all Cyt1 and Cyt2 protein between the sheet formed by β 4, β 6- β 8, and helices α 3- α 5 (Cyt1Aa numbering). In the case of Evf, a palmitate covalently bound by a cysteine is found in a hydrophobic pocket.



Sequence alignment of Cyt from D. dadantii, Cyt1 and Cyt2 family members. Residues colored by their hydrophobicity properties from red (hydrophobic) to blue (hydrophilic) and by conservation. **The conserved** *residues forming the cavity are marked by black asterisks*.



Dickeya toxin cluster evolved in a multistep process involving a double tandemduplication step, giving birth to the CytBC clade (proteins shorter than the two other groups, ≈200 residues vs ≈220 residues for CytA and CytD).

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Reference: New Cyt-like 8-endotoxins from *Dickeya dadantii*: structure and aphicidal activity. **Loth et al. submitted**

<u>Conclusion</u> : CytC adopts a cytolysin fold and can be structurally classified as a Cyt2-like protein. The NMR ensemble of structures contains two distinct and equally populated conformations in solution, which could be in agreement with the Cyt protein pore-forming mechanism. Moreover, the identification of a putative lipid binding pocket in CytC NMR structure, which has been maintained in two clades (*evf* and *Dickeya* clades), and probably in most members of the Cyt-toxin family, could support the importance of this putative lipid binding cavity for the mechanism of action of the whole family. Our integrative approach provided significant insights into the evolutionary and functional history of *D. dadantii* Cyt toxins, which appears to be interesting leads for biopesticides.