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Vection des bactéries par les insectes : la cuticule importe t'elle ?

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Vection des bactéries par les insectes : la cuticule importe t'elle ?

Rahbé, Yvan
Guschinskaya, Natalia
Monsion, Baptiste
Uzest, Marilyne



MAP

- Part A: Panoramic
- Part B: Experimental

From Cuticle to cuticular proteins

Epicuticle: the outermost envelope (lipids + polyphenol + few proteins)

Exocuticle: (or inner epicuticle): lipids + proteins

Endocuticle: chitin + matrix of proteins + lipids + pigments and N-acylcatecholamines
→ cross-linking

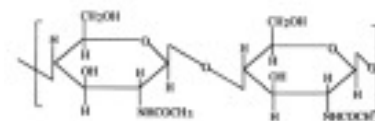
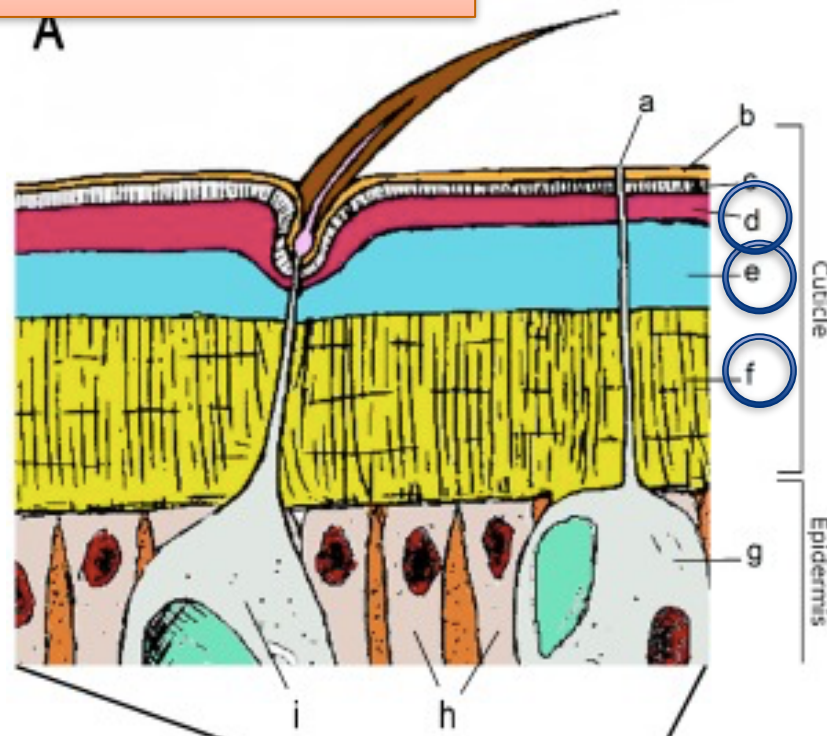
→ The composition of the insect cuticle with respect to protein content can be highly variable

Building Parts of insect Cuticle :

a: Glandular duct
b: cement
c: wax

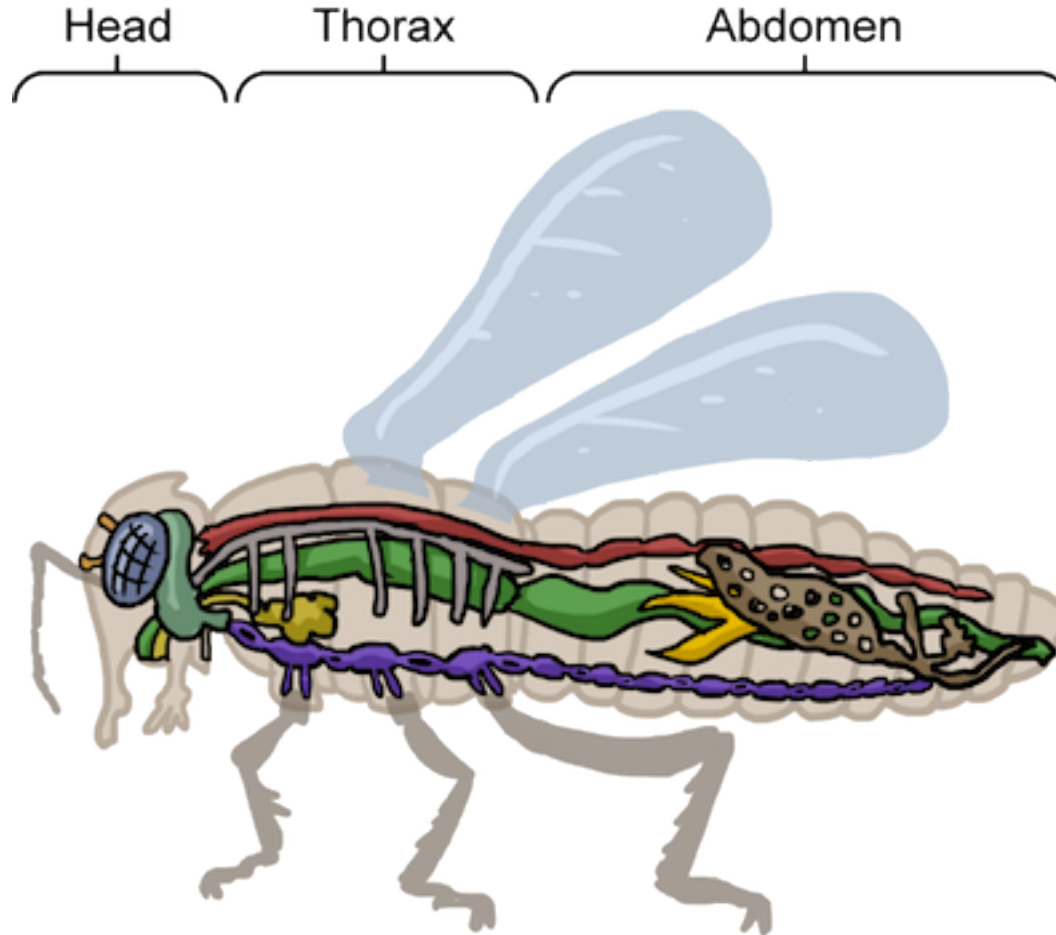
d: epicuticle
e: exocuticle
f: endocuticle

g: glandular cell
h: epidermal cell

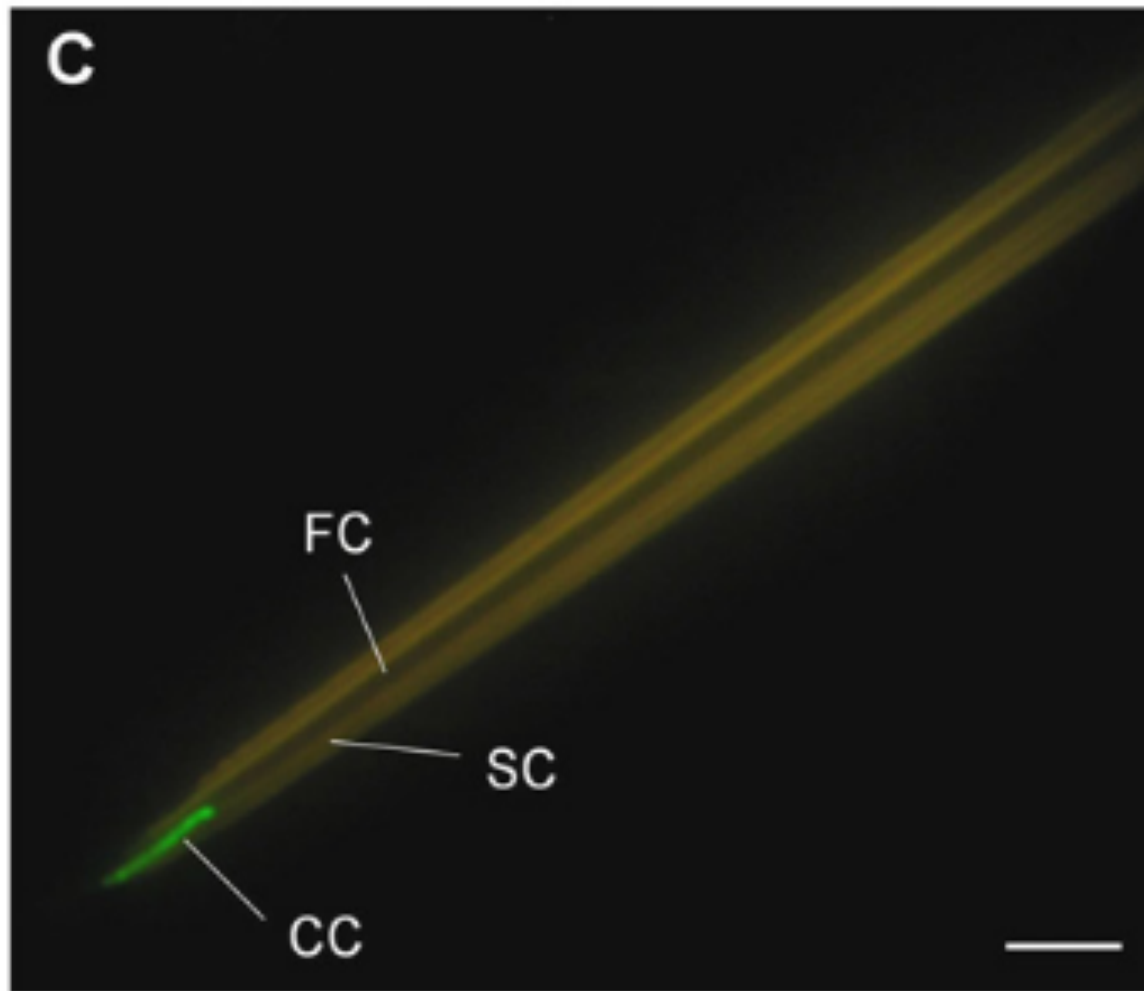


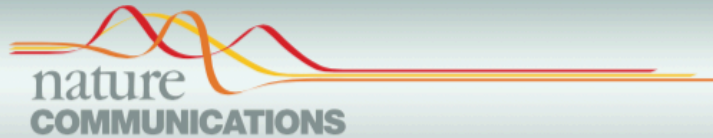
Chitin





<https://www.shmoop.com/animal-evolution-diversity/land-animals.html>





ARTICLE

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OPEN

Bactericidal activity of black silicon

Elena P. Ivanova¹, Jafar Hasan¹, Hayden K. Webb¹, Gediminas Gervinskas^{2,3}, Saulius Juodkazis^{2,3}, Vi Khanh Truong¹, Alex H.F. Wu⁴, Robert N. Lamb⁴, Vladimir A. Baulin⁵, Gregory S. Watson⁶, Jolanta A. Watson⁶, David E. Mainwaring¹ & Russell J. Crawford¹

Black silicon is a synthetic nanomaterial that contains high aspect ratio nanoprotusions on its surface, produced through a simple reactive-ion etching technique for use in photovoltaic applications. Surfaces with high aspect-ratio nanostructures are also common in the natural world, for example, the wings of the dragonfly *Diplacodes bipunctata*. Here we show that the nanoprotusions on the surfaces of both black silicon and *D. bipunctata* wings form hierarchical structures through the formation of clusters of adjacent nanoprotusions. These structures generate a mechanical bactericidal effect, independent of chemical composition. Both surfaces are highly bactericidal against all tested Gram-negative and Gram-positive bacteria, and endospores, and exhibit estimated average killing rates of up to $\sim 450,000$ cells $\text{min}^{-1} \text{cm}^{-2}$. This represents the first reported physical bactericidal activity of black silicon or indeed for any hydrophilic surface. This biomimetic analogue represents an excellent prospect for the development of a new generation of mechano-responsive, antibacterial nanomaterials.

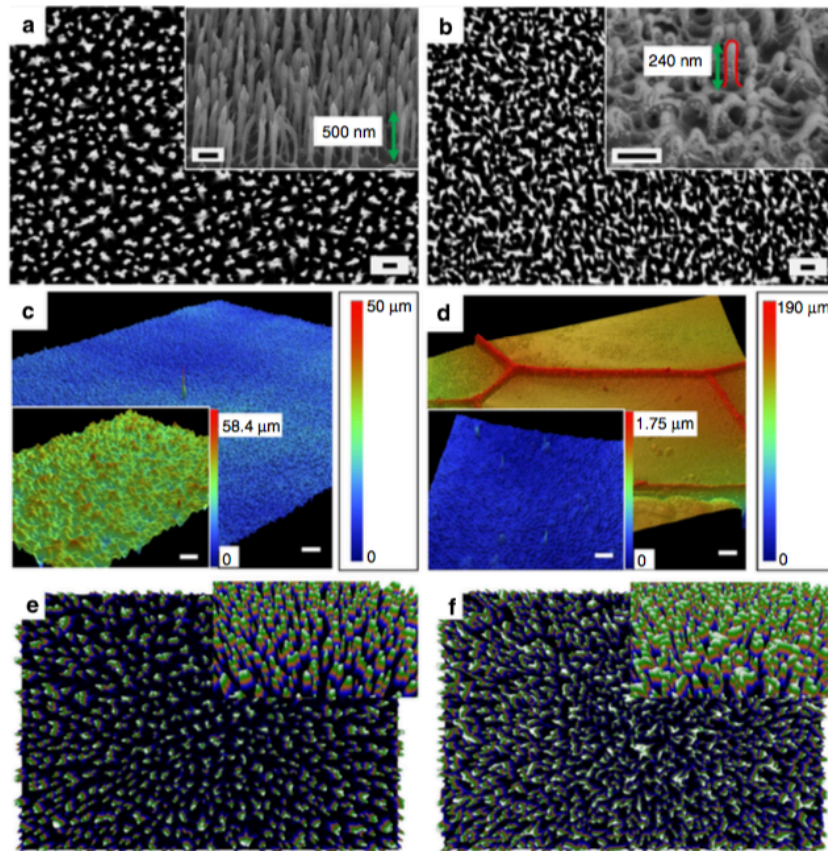


Figure 1 | Characterization of black silicon and *D. bipunctata* wings. Scanning electron micrographs of the upper surface of (a) bSi and (b) dragonfly

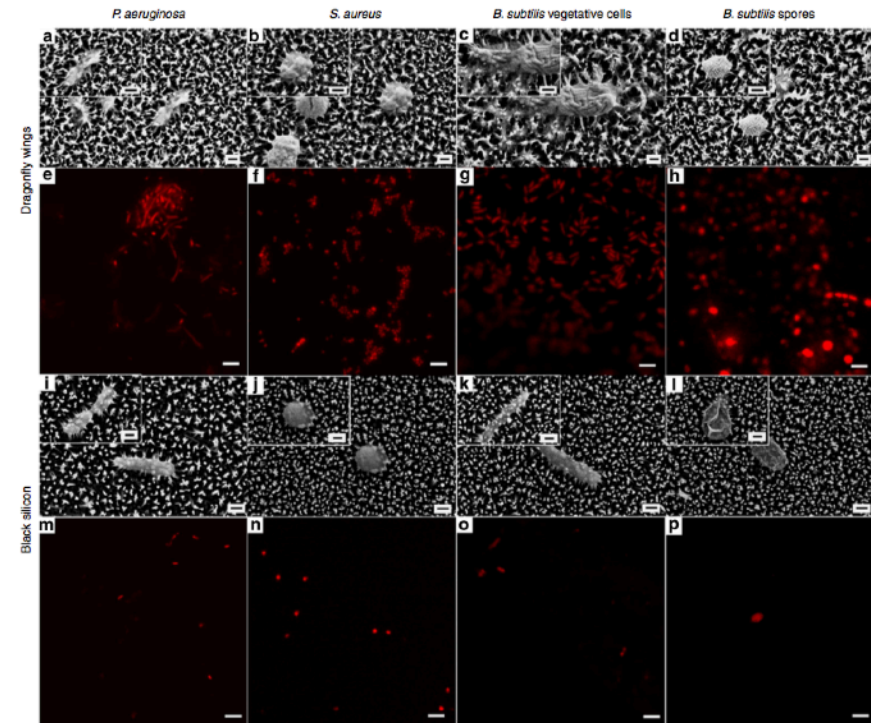
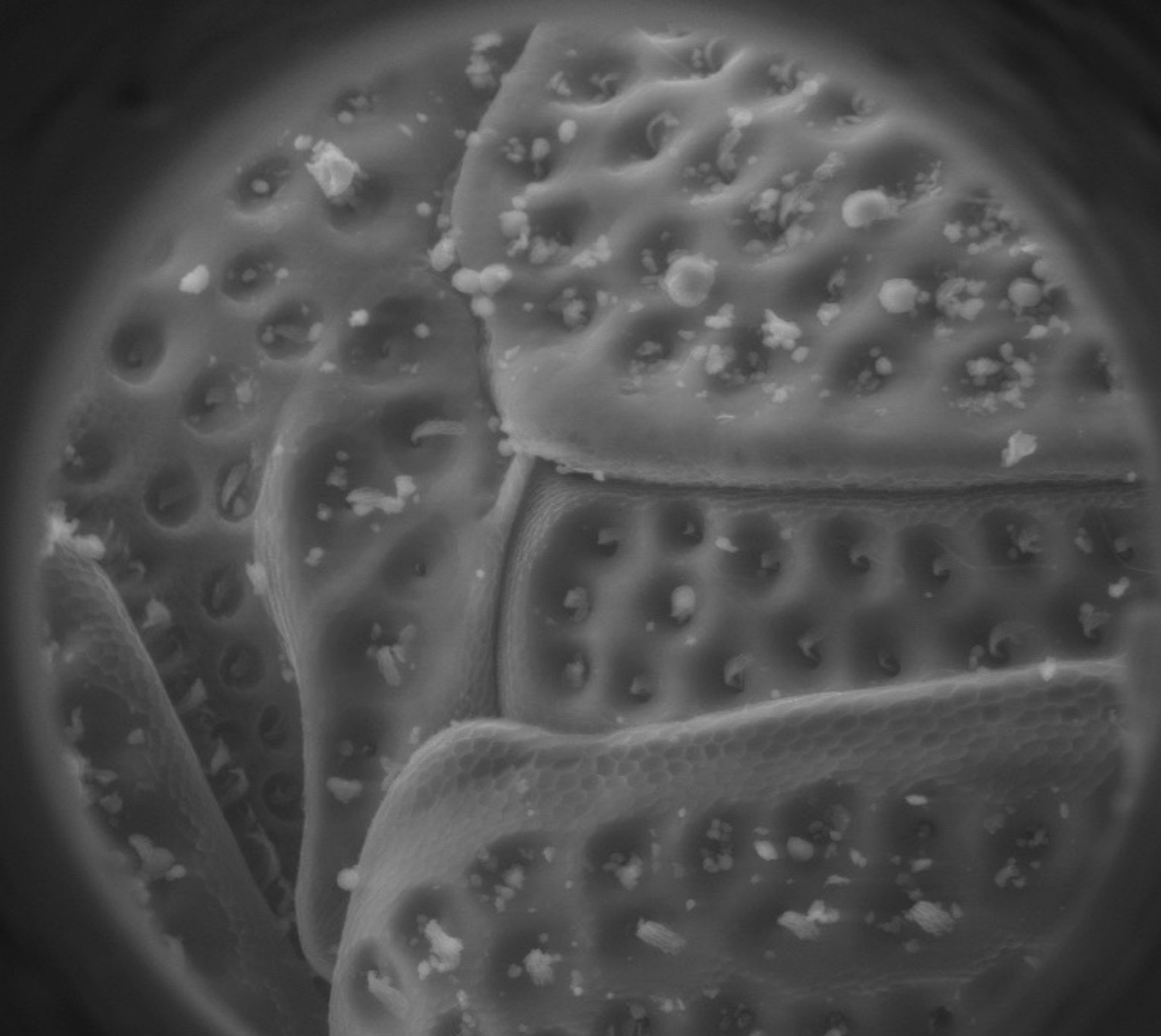


Figure 2 | Cell morphology on dragonfly wings and black silicon. SEM images of *P. aeruginosa*, *S. aureus*, *B. subtilis* vegetative cells and *B. subtilis* spores appear to be significantly disrupted through interaction with both the dragonfly wing (a-d) and bSi (i-l). Scale bars, 200 nm. Confocal laser scanning micrographs confirm that disruption by dragonfly wing (e-h) and bSi (m-p) was lethal to the cells; non-viable bacterial cells and spores were stained with propidium iodide (red), whereas the living cells were stained with SYTO 9 (green). All cells appeared red, indicating the high efficiency of surfaces in inactivating the bacteria. Scale bars, 5 μm.

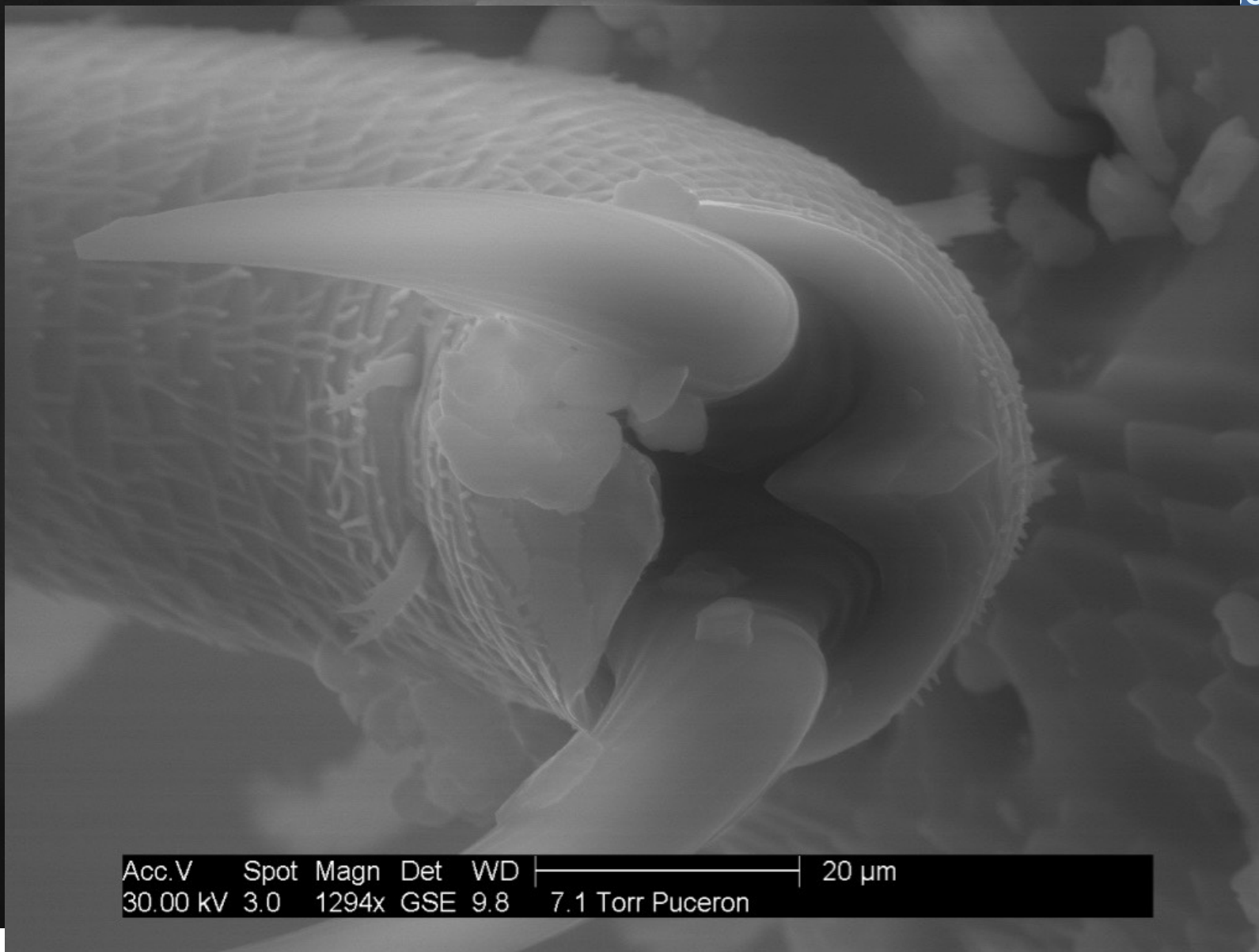


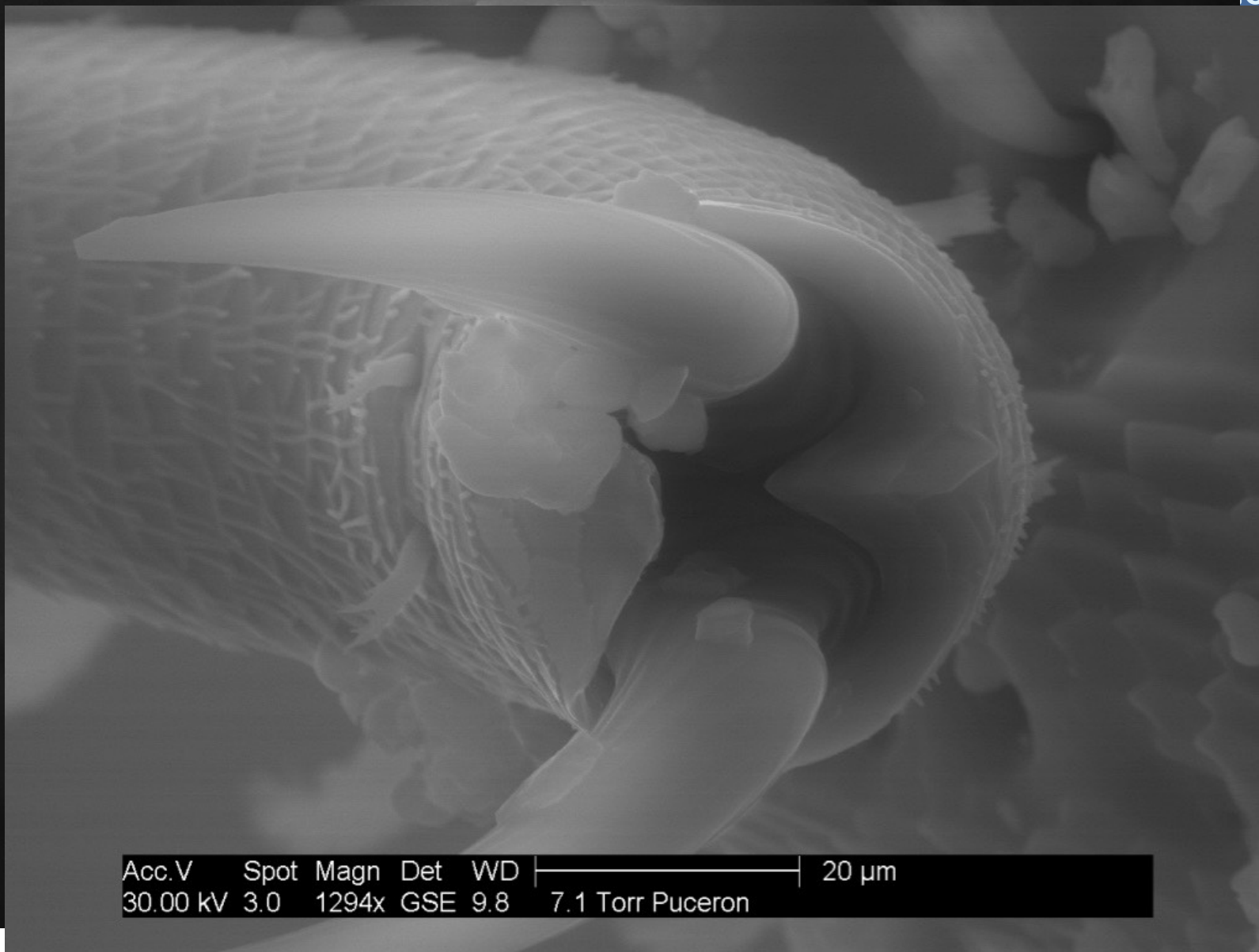


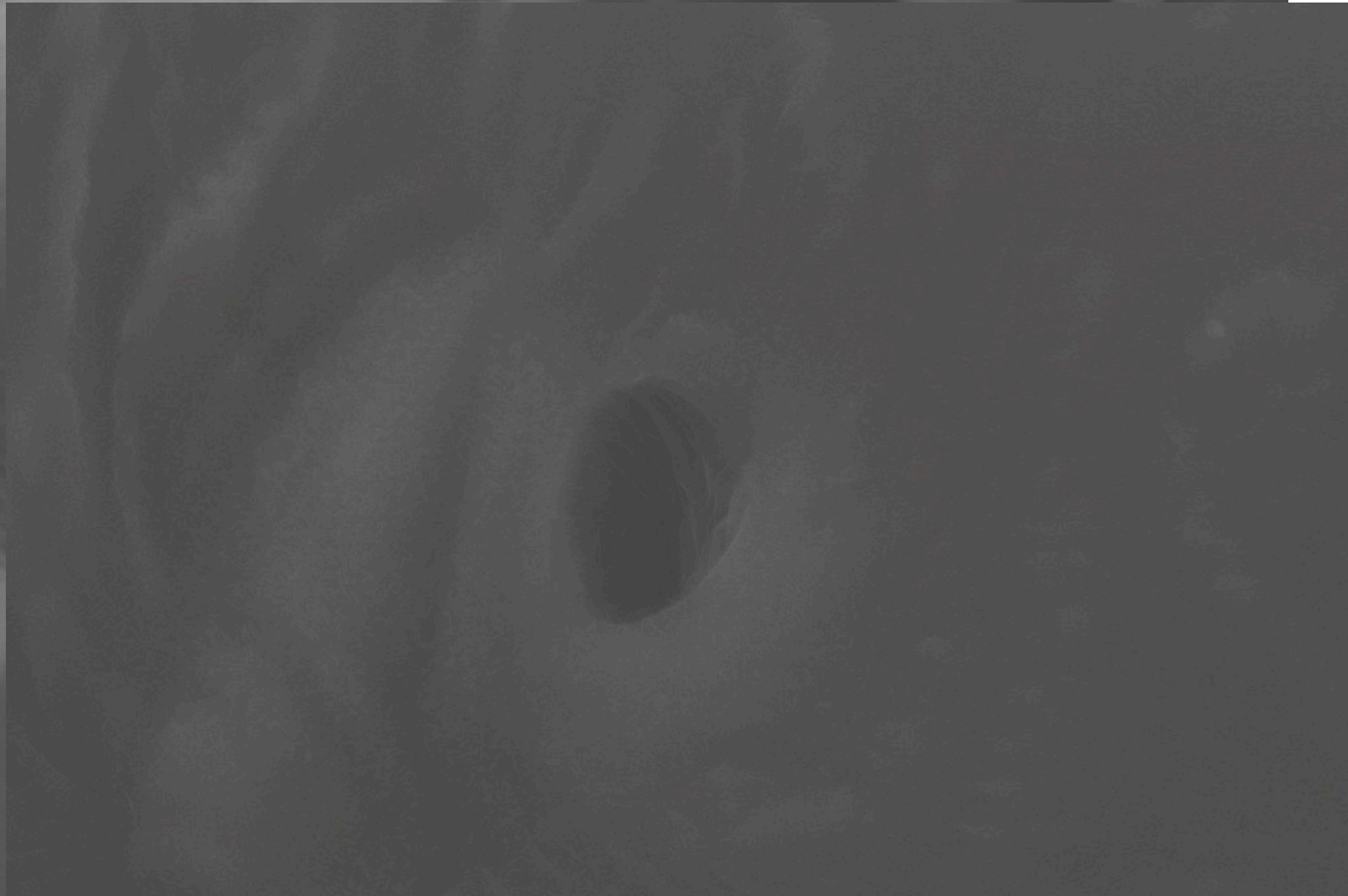
Acc.V Spot Magn Det WD | 200 µm
30.00 kV 3.0 152x GSE 9.7 5.7 Torr



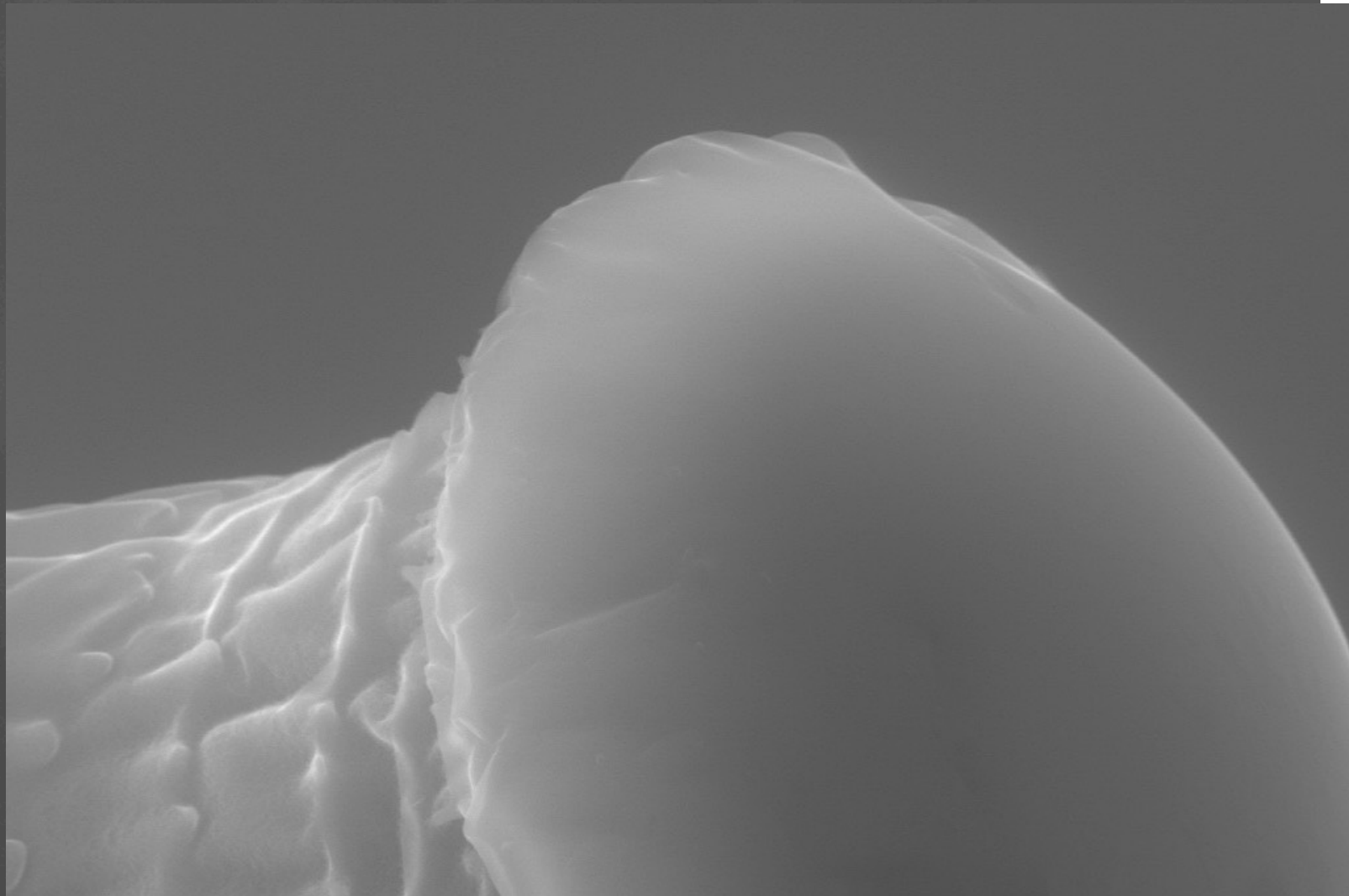
Acc.V Spot Magn Det WD |-----| 100 µm
30.00 kV 3.0 162x GSE 10.6 6.9 Torr Puceron



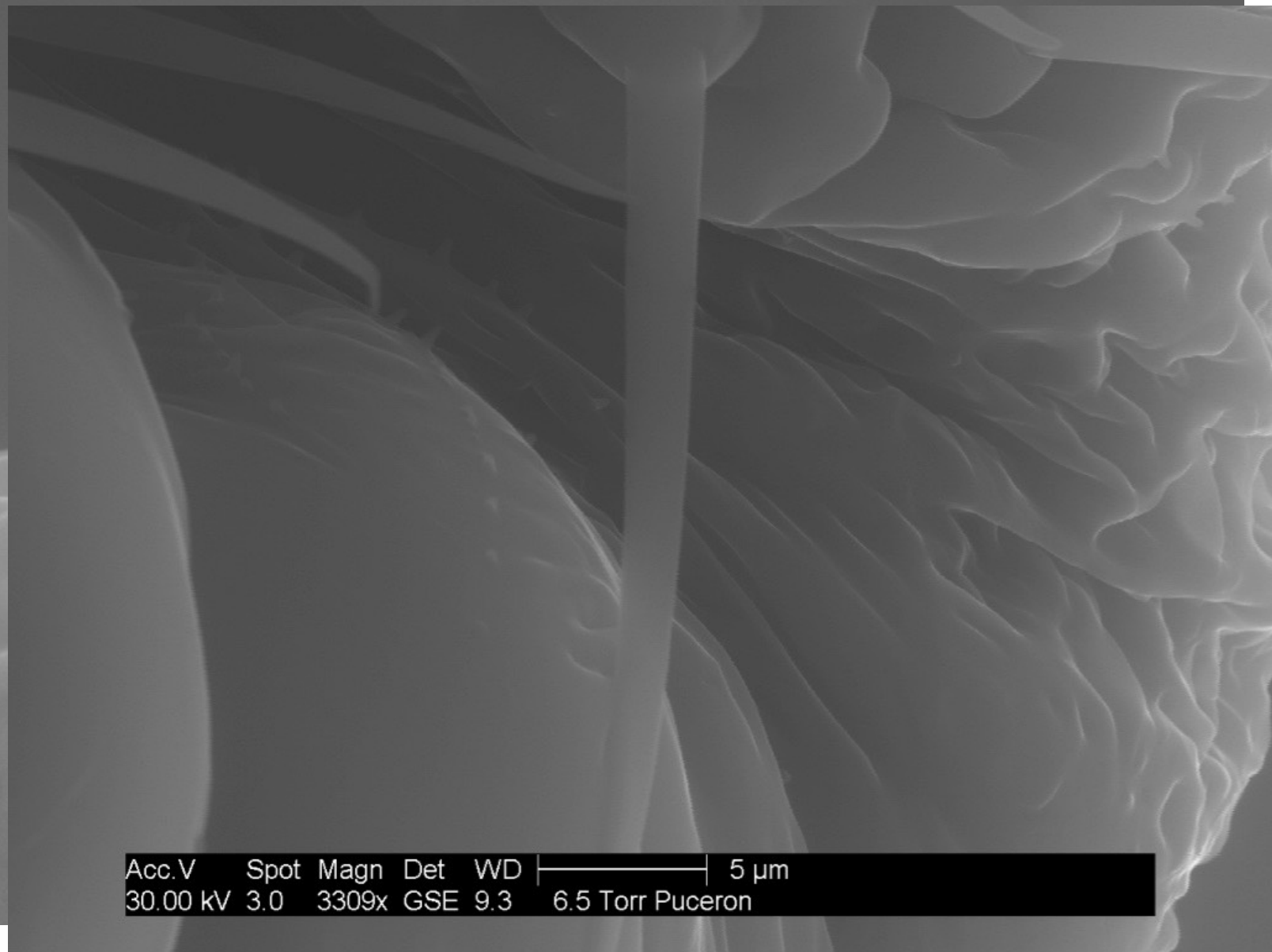




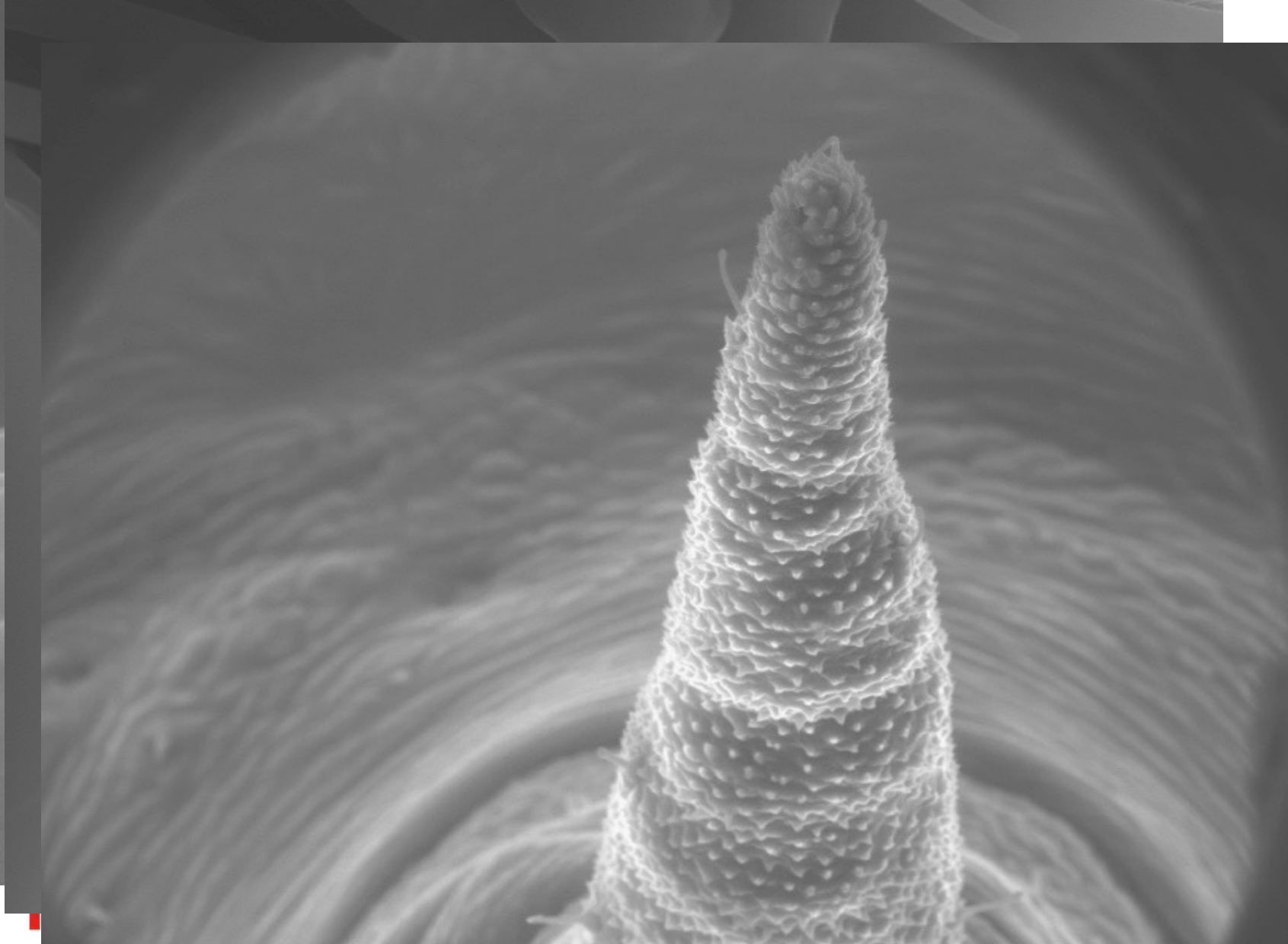
Acc.V	Spot	Magn	Det	WD	----- 20 μm	
30.00 kV	3.0	1213x	GSE	12.1	6.5 Torr	Puceron

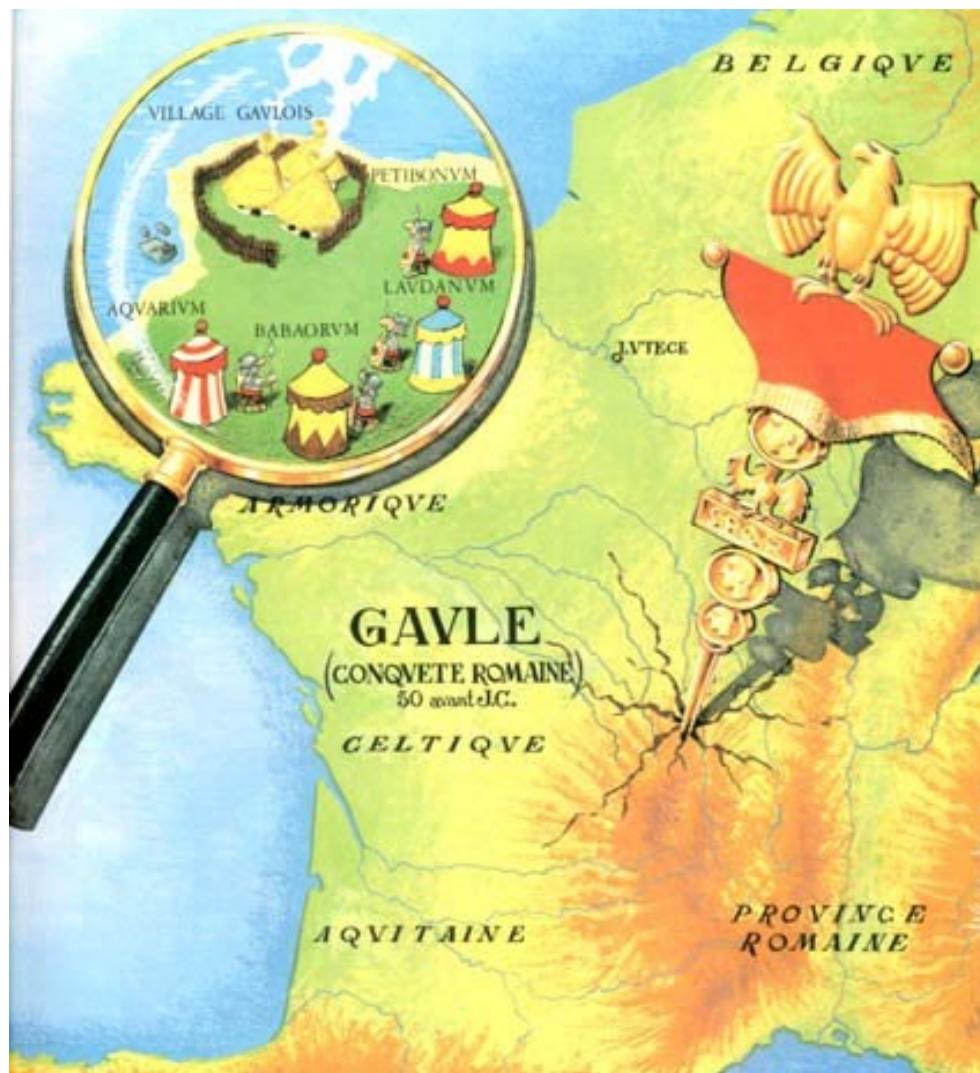


Acc.V	Spot	Magn	Det	WD	10 μm
30.00 kV	3.0	2426x	GSE	11.3	6.5 Torr Puceron



Acc.V	Spot	Magn	Det	WD	5 μ m
30.00 kV	3.0	3309x	GSE	9.3	6.5 Torr Puceron





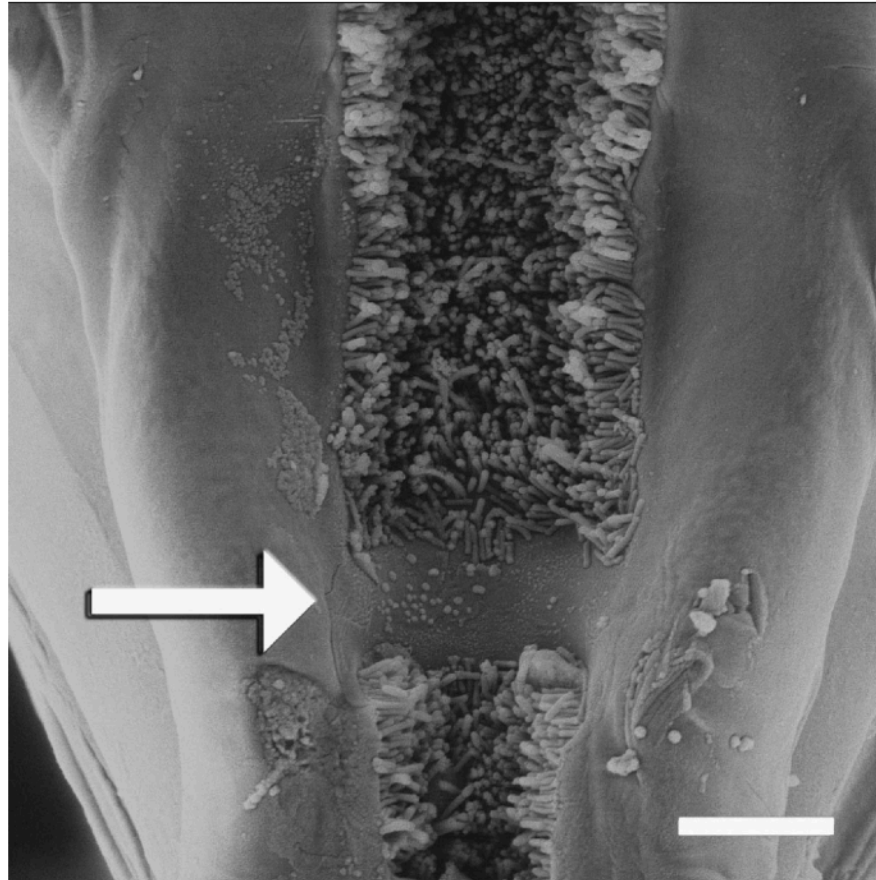


Fig. 5. Hypopharynx of *G. atropunctata* showing region always free of *X. fastidiosa* (arrow). This region matches the location of the precibarial valve on the epipharynx. Absence of cells may be associated with movement of the valve or contact to structures associated with the valve present in the epipharynx. Bar, 10 μm .



Factors Affecting the Initial Adhesion and Retention of the Plant Pathogen *Xylella fastidiosa* in the Foregut of an Insect Vector

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Citrus Research and Education Center, Department of Entomology and Nematology, University of Florida, IFAS, Lake Alfred, Florida, USA^a; Department of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, California, USA^b

Vector transmission of bacterial plant pathogens involves three steps: pathogen acquisition from an infected host, retention within the vector, and inoculation of cells into susceptible tissue of an uninfected plant. In this study, a combination of plant and artificial diet systems were used to determine the importance of several genes on the initial adhesion and retention of the bacterium *Xylella fastidiosa* to an efficient insect vector. Mutant strains included fimbrial (*fimA* and *pilB*) and afimbrial (*hxfA* and *hxfB*) adhesins and three loci involved in regulatory systems (*rpfF*, *rpfC*, and *cgsA*). Transmission assays with variable retention time indicated that HxfA and HxfB were primarily important for early adhesion to vectors, while FimA was necessary for both adhesion and retention. The long pilus protein PilB was not deficient in initial adhesion but may be important for retention. Genes upregulated under the control of *rpfF* are important for both initial adhesion and retention, as transmission rates of this mutant strain were initially low and decreased over time, while disruption of *rpfC* and *cgsA* yielded trends similar to that shown by the wild-type control. Because induction of an *X. fastidiosa* transmissible state requires pectin, a series of experiments were used to test the roles of a polygalacturonase (*pglA*) and the pectin and galacturonic acid carbohydrates on the transmission of *X. fastidiosa*. Results show that galacturonic acid, or PglA activity breaking pectin into its major subunit (galacturonic acid), is required for *X. fastidiosa* vector transmission using an artificial diet system. This study shows that early adhesion and retention of *X. fastidiosa* are mediated by different factors. It also illustrates that the interpretation of results of vector transmission experiments, in the context of vector-pathogen interaction studies, is highly dependent on experimental design.



MPMI Vol. 29, No. 7, 2016, pp. 535–544. <http://dx.doi.org/10.1094/MPMI-02-16-0032-R>

Blocking the Transmission of a Noncirculative Vector-Borne Plant Pathogenic Bacterium

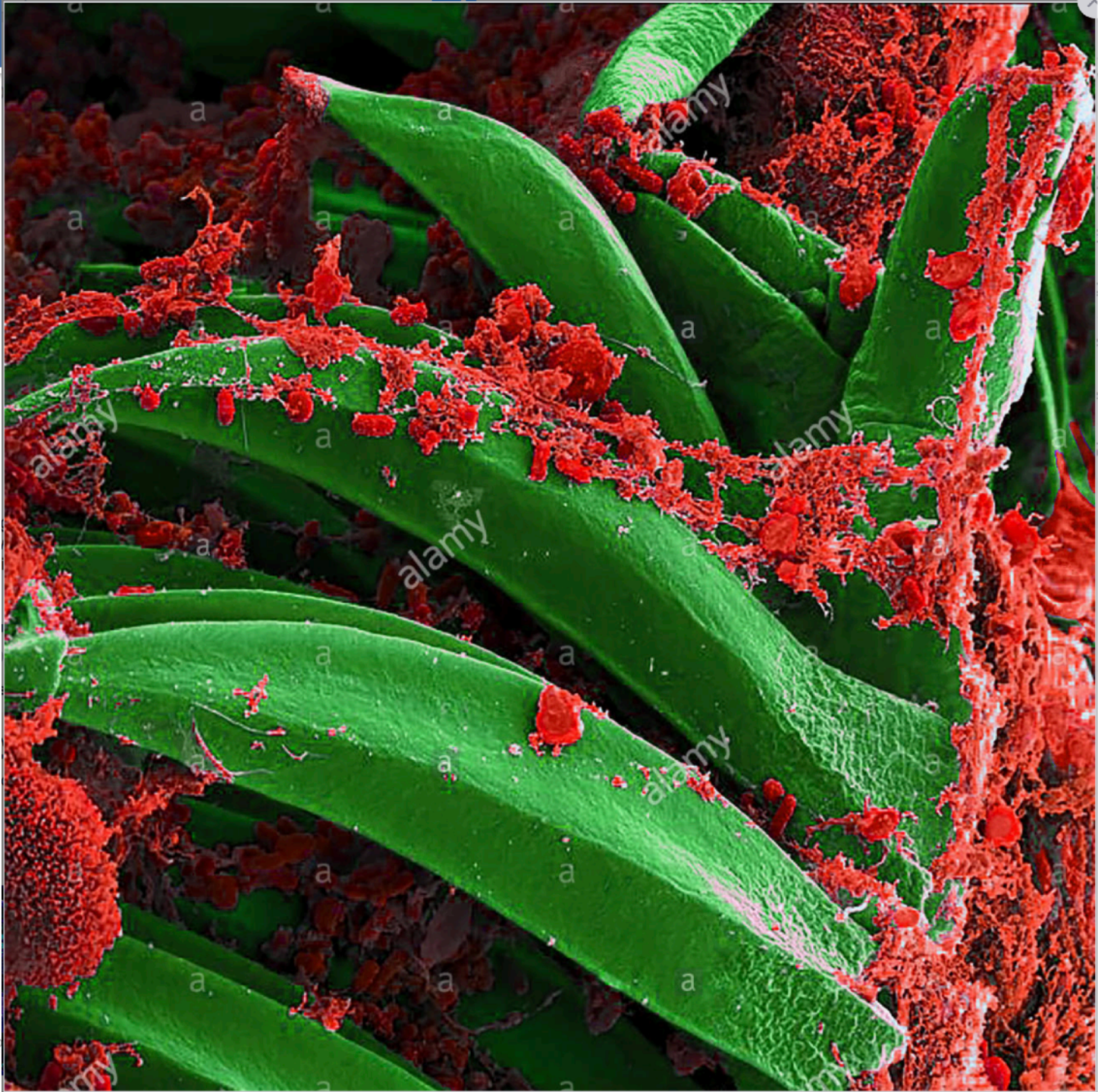
Oui, des bactéries peuvent s'accrocher spécifiquement à des territoires cuticulaires définis,

- par certains déterminants bactériens identifiés (eg PD1764, HxfB) et
- par des déterminants insectes ciblés (chitine)

The successful control of insect-borne plant pathogens is often difficult to achieve due to the ecologically complex interactions among pathogens, vectors, and host plants. Disease management often relies on pesticides and other approaches that have limited long-term sustainability. To add a new tool to control vector-borne diseases, we attempted to block the transmission of a bacterial insect-transmitted pathogen, the bacterium *Xylella fastidiosa*, by disrupting bacteria–insect vector interactions. *X. fastidiosa* is known to attach to and colonize the cuticular surface of the mouthparts of vectors; a set of recombinant peptides was generated and the chemical affinities of these peptides to chitin and related carbohydrates was assayed in vitro. Two candidates, the *X. fastidiosa* hypothetical protein PD1764 and an N-terminal region of the hemagglutinin-like protein B (HxfB) showed affinity for these substrates. These proteins were provided to vectors via an artificial diet system in which insects acquire *X. fastidiosa*, followed by an inoculation access period on plants under greenhouse conditions. Both PD1764 and HxfAD1-3 significantly blocked transmission. Furthermore, bacterial populations within insects over a 10-day period demonstrated that these peptides inhibited cell adhesion to vectors but not bacterial multiplication, indicating that the mode of action of these peptides is restricted to limiting cell adhesion to insects, likely via competition for adhesion sites. These results open a new venue in the search for sustainable disease-control strategies that are pathogen specific and may have limited nontarget effects.

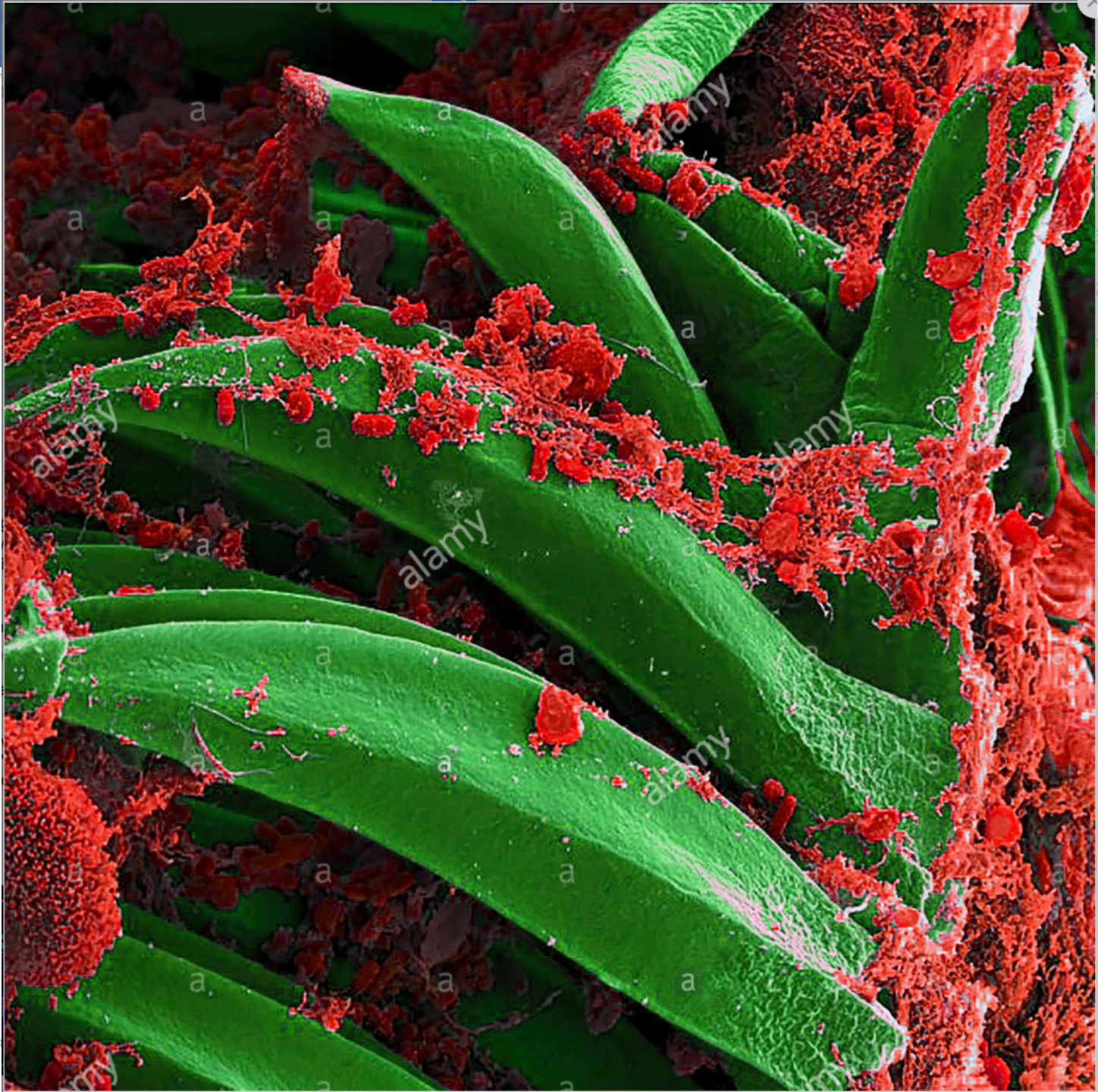


Autres insectes ?





Scanning electron micrograph of *Yersinia pestis* on proventricular spines of *Xenopsylla cheopis*





Et les Sternorrhynques ?



Acquisition and Transmission of *Agrobacterium* by the Whitefly *Bemisia tabaci*

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Received 15 July 1993. Accepted 31 August 1994.

Whiteflies transmit many different plant pathogens. We show here that the whitefly *Bemisia tabaci* can also acquire *Agrobacterium tumefaciens* from liquid cultures and from crown galls, and transmit it to plants. Cloned tomato yellow leaf curl virus (TYLCV) DNA and *A. tumefaciens* tumor-inducing functions were used as reporter genes. Whiteflies were fed through membranes on cultures of *Agrobacterium* At::pTY4 containing a dimeric copy of an infectious TYLCV genomic clone between the T-DNA borders of a binary vector. One hour of feeding was sufficient for the insects to be able to transmit TYLCV to tomato test plants. Infectious *Agrobacterium* was recovered from whiteflies that fed on At::pTY4 cultures, indicating that bacteria was acquired and remained intact in the insects. Whiteflies also acquired the virulent *A. tumefaciens* strain C58 from crown galls and induced tumors in tomato test plants. PCR and Southern blot analyses indicated that the target plant tissues were transformed. These results show that an insect can transfer foreign genes to plants by acquiring and delivering transforming bacteria.

1987). The best studied group is the geminiviruses and the most studied vector is the tobacco whitefly, *Bemisia tabaci* Gennadius. Only geminiviruses are transmitted in a persistent-circulative way by the insect, i.e., they can be maintained and transmitted by the vector for several days to life. Acquisition and retention of two *B. tabaci*-transmitted geminiviruses have been studied using molecular tools: squash leaf curl virus (SLCV; Polston *et al.* 1990) and tomato yellow leaf curl virus (TYLCV; Zeidan and Czosnek 1991).

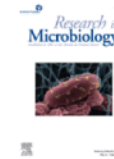
We have shown previously that the whitefly, *B. tabaci*, can feed through membranes on sucrose solutions containing TYLCV particles and transmit the TYLCV disease to test plants (Czosnek *et al.* 1988a). In this paper we report that the whitefly *B. tabaci* can also acquire genetic information from cultures of *Agrobacterium* and from crown galls, and transmit this information to test plants. The TYLCV genome and the *A. tumefaciens* Ti plasmid tumor-inducing functions were used as reporter genes. This study may help to extend our knowledge of the variety of interactions between whiteflies and pathogens.

RESULTS



Research in Microbiology

Volume 165, Issue 2, February–March 2014, Pages 77-81



Bacterial endosymbionts in field-collected samples of *Trialeurodes* sp. nr. *abutiloneus* (Hemiptera: Aleyrodidae)

Bodil N. Cass ^a✉, Netta Mozes-Daube ^b✉, Lilach Iasur-Kruh ^b✉, Elizabeth C. Bondy ^c✉, Suzanne E. Kelly ^c✉, Martha S. Hunter ^c✉, Einat Zchori-Fein ^b✉

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<https://doi.org/10.1016/j.resmic.2014.01.005>

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Abstract

Facultative bacterial endosymbionts are common, influential associates of arthropods, yet their movement among host species has not been well documented. Plant-mediated transmission of *Rickettsia* has been shown for the whitefly *Bemisia tabaci*. *Bemisia tabaci* in USA cotton fields harbors the secondary symbionts *Rickettsia* and *Hamiltonella*, and co-occurs with *Trialeurodes* sp. nr. *abutiloneus* whiteflies. To determine whether symbionts may be shared, the microbial diversity of these whiteflies on cotton across the USA was analyzed. *Trialeurodes* sp. nr. *abutiloneus* bore *Portiera*, *Pseudomonas*, *Serratia*, *Arsenophonus* and *Wolbachia*. No *Rickettsia* or *Hamiltonella* were detected. These results provide no evidence for horizontal transmission of symbionts between these whitefly genera.

Keywords

Bacteria; Distribution pattern; Symbiosis; Transmission routes



Et les pucerons ?

Acquisition

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2006, p. 1956–1965
 0099-2240/06/\$08.00+0 doi:10.1128/AEM.72.3.1956–1965.2006
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Vol. 72, No. 3

The Phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) Is a Pathogen of the Pea Aphid†

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Received 6 October 2005/Accepted 4 January 2006

Dickeya dadantii (*Erwinia chrysanthemi*) is a phytopathogenic bacterium causing soft rot diseases on many crops. The sequencing of its genome identified four genes encoding homologues of the Cyt family of insecticidal toxins from *Bacillus thuringiensis*, which are not present in the close relative *Pectobacterium carotovorum* subsp. *atrosepticum*. The pathogenicity of *D. dadantii* was tested on the pea aphid *Acyrtosiphon pisum*, and the bacterium was shown to be highly virulent for this insect, either by septic injury or by oral infection. The lethal inoculum dose was calculated to be as low as 10 ingested bacterial cells. A *D. dadantii* mutant with the four cytotoxin genes deleted showed a reduced per os virulence for *A. pisum*, highlighting the potential role of at least one of these genes in pathogenicity. Since only one bacterial pathogen of aphids has been previously described (*Erwinia aphidicola*), other species from the same bacterial group were tested. The pathogenic trait for aphids was shown to be widespread, albeit variable, within the phytopathogens, with no link to phylogenetic positioning in the *Enterobacteriaceae*. Previously characterized gut symbionts from thrips (*Erwinia/Pantoea* group) were also highly pathogenic to the aphid, whereas the potent entomopathogen *Photobacterium luminescens* was not. *D. dadantii* is not a generalist insect pathogen, since it has low pathogenicity for three other insect species (*Drosophila melanogaster*, *Sitophilus oryzae*, and *Spodoptera littoralis*). *D. dadantii* was one of the most virulent aphid pathogens in our screening, and it was active on most aphid instars, except for the first one, probably due to anatomical filtering. The observed difference in virulence toward apterous and winged aphids may have an ecological impact, and this deserves specific attention in future research.



GroEL from the endosymbiont *Buchnera aphidicola* betrays the aphid by triggering plant defense

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Contributed by Steven P. Briggs, April 28, 2014 (sent for review March 16, 2014)

Aphids are sap-feeding plant pests and harbor the endosymbiont *Buchnera aphidicola*, which is essential for their fecundity and survival. During plant penetration and feeding, aphids secrete saliva that contains proteins predicted to alter plant defenses and metabolism. Plants recognize microbe-associated molecular patterns and induce pattern-triggered immunity (PTI). No aphid-associated molecular pattern has yet been identified. By mass spectrometry, we identified in saliva from potato aphids (*Macrosiphum euphorbiae*) 105 proteins, some of which originated from *Buchnera*, including the chaperonin GroEL. Because GroEL is a widely conserved bacterial protein with an essential function, we tested its role in PTI. Applying or infiltrating GroEL onto *Arabidopsis* (*Arabidopsis thaliana*) leaves induced oxidative burst and expression of PTI early marker genes. These GroEL-induced defense responses required the known coreceptor BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1. In addition, in transgenic *Arabidopsis* plants, inducible expression of *groEL* activated PTI marker gene expression. Moreover, *Arabidopsis* plants expressing *groEL* displayed reduced fecundity of the green peach aphid (*Myzus persicae*), indicating enhanced resistance against aphids. Furthermore, delivery of GroEL into tomato (*Solanum lycopersicum*) or *Arabidopsis* through *Pseudomonas fluorescens*, engineered to express the type III secretion system, also reduced potato aphid and green peach aphid fecundity, respectively. Collectively our data indicate that GroEL is a molecular pattern that triggers PTI.

likely to be secreted (10). However, direct profiling of the aphid salivary proteome using mass spectrometry (MS) identified only about three dozen secreted proteins (11–16). This apparent discrepancy is likely due to the scarcity of saliva secreted by aphids in vitro.

To characterize the aphid salivary proteome, saliva was collected from a large number of potato aphids (*Macrosiphum euphorbiae*) and was profiled using high-throughput proteomics-based liquid chromatography, nanoelectrospray ionization, and tandem MS. Here we report the identification of aphid salivary proteins among which are proteins of the endosymbiont *Buchnera* origin and the recognition of one of these endosymbiont proteins, the chaperonin GroEL, by the plant innate immune system.

Results and Discussion

Aphid Saliva Contains Large Numbers of Proteins. To characterize the aphid salivary proteome, liquid and gelling saliva were collected in vitro in water from about 100,000 potato aphids. Proteins in the two types of saliva were profiled using MS. To identify salivary proteins of aphid origin, the MS spectra were searched against a predicted potato aphid proteome, which was derived from transcriptome data, as the genome of the potato aphid has not been sequenced. We identified a total of 94 aphid proteins in combined gelling and liquid saliva ([Dataset S1](#) and [SI Appendix, Text](#)). The great majority of these proteins were

Erwinia iniecta sp. nov., isolated from Russian wheat aphid (*Diuraphis noxia*)

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Aphids are sap-sucking insects. They penetrate plant tissues using a special mouthpart – the stylet – and they probe between the plant cell layers to find sieve elements. Once stylets penetrate the sieve elements, they alternately inject saliva and suck the plant sap (for a review, see Miles, 1999). This process for obtaining nutrients establishes an intimate and long-term interaction between the aphids and the host plant. In a few cases, aphids are proposed to vector or deliver plant-pathogenic bacteria into the plants

Abbreviations: ANI, average nucleotide identity; MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains B120^T, B137, B149 and B150 are KM870781–KM870784, respectively. The accession numbers for the draft genome sequences of B120^T and B149 are JRXF00000000 and JRXE00000000, respectively.

Four supplementary tables and a supplementary figure are available with the online Supplementary Material.

via their stylets (Plurad *et al.*, 1965; Watanabe *et al.*, 1996; Stavrinides *et al.*, 2009).

In a previous study, we repeatedly and consistently isolated members of the *Enterobacteriaceae* from sterile artificial diets (15 % aqueous sucrose, pH 7.2) fed on by Russian wheat aphids [*Diuraphis noxia* (Kurdjumov)] (T. Campillo, L. van Eck, E. Luna, N. Lapitan, N. Tisserat and J. E. Leach, unpublished results). The bacteria were isolated only from artificial diets exposed to probing of *D. noxia* stylets through a Parafilm membrane; no bacteria were isolated from sterile diets that had not been exposed to aphids. In addition, bacteria were isolated from crushed aphid bodies and from wheat exposed to *D. noxia* feeding. Based on 16S rRNA gene sequences of 30 diverse isolates from these various origins, most of the bacteria grouped within the *Enterobacteriaceae*.

Of the bacteria isolated from aphid bodies or artificial diets or wheat after feeding by aphids, we found a subset of isolates that belong to the *Erwinia*–*Pantoea* clade. While members of the genera *Erwinia* and *Pantoea* are most commonly



Oui, des bactéries ont **vraisemblablement** le potentiel de se lier **spécifiquement** à des **territoires cuticulaires**, notamment internes des pièces buccales et de l'intestin antérieur (**stylets & foregut**), dans d'autres modèles que la maladie de Pierce

Exploring the interaction between bacteria and insect cuticular proteins by peptide Arrays using the pea aphid (*Acyrtosiphon pisum*) model.

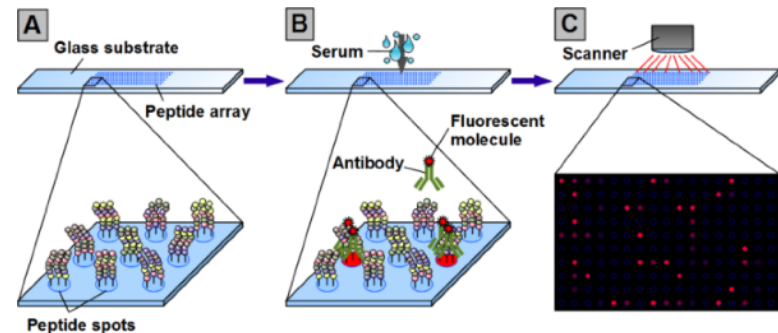
Stagiaire: **Hoang Thong KIEU**,
M2 biochimie-biologie moléculaire

Laboratoire d'accueil: **MAP** UMR5240 CNRS UCBL INSA de Lyon

Encadrant: **Yvan RAHBE**, DR INRA

Natalia GUSCHINSKAYA, Post doct.

Collaboration: **Guy Condemine**, DR CNRS

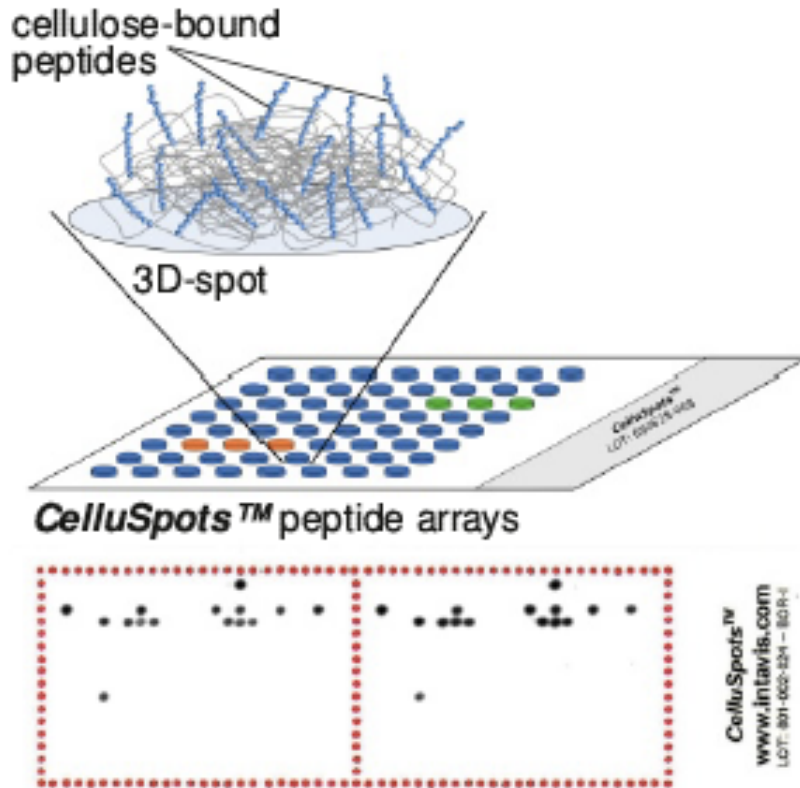




Main objective

**Screening the interactions, at the molecular level,
between the cuticular proteins of
Acyrtosiphon pisum
and the phytopathogenic bacteria Dickeya dadantii,
by peptide arrays**

What is a peptide array ?

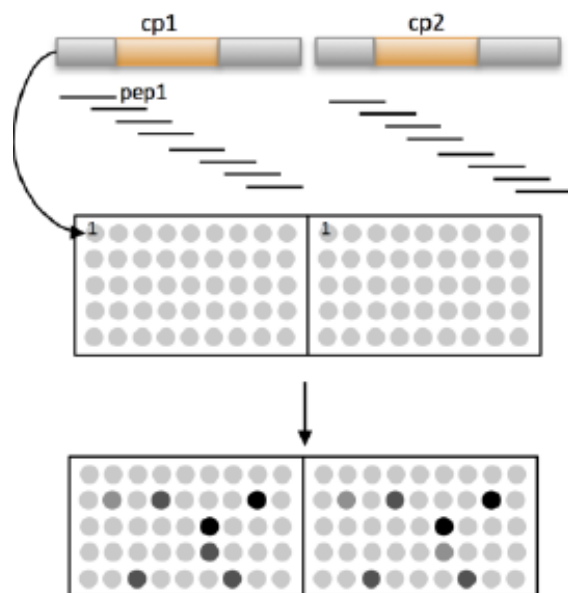
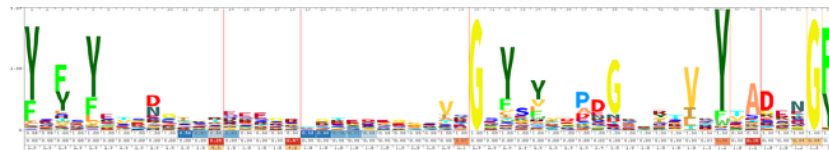


- **Size: 76 × 26 mm** (microscope slide)
- **Peptides are covalently bound to cellulose via C_{ter}.**
- **18 aa peptides immobilised on the spots.**
- **Spots: 2 × 384 spots** (technical repeat)
- **Distance between spots: 1,2 mm.**
- **Spot diameter 0.8 mm.**
- **Cellulose mesh of : 8 – 10 μm.**
- **2 versions: V1 and V2** (peptides / proteins)

CelluSpots™ peptide arrays - Intavis

Array design

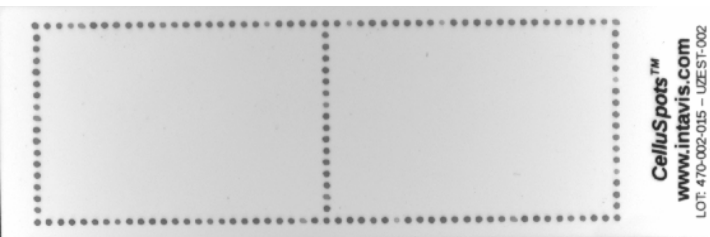
- 143 Cuticular proteins in the genome of the pea aphid
Belonging to 5 classes, among which the largest is the CPR family (PFAM PF00379), or « RR motif family »



- 3 Subclasses: RR1, RR2 and Unclassified CPRs
- V1 contains a complete set of RR2 proteins
(core set, full coverage)
- V2 contains:
 - All RR1 (full set, full coverage)
 - Remaining RR2
(partial set, partial coverage)
 - Remaining, = Unclassified CPR
(partial set, partial coverage)
- 18 AA peptides with 3 AA overlap
- Peptides are immobilized as 2 duplicates in array
- Example of chemiluminescence detection of hybridization
(HRP, peroxidase in Luminata luminol-based system)



Detection method



Arrays V1
& V2

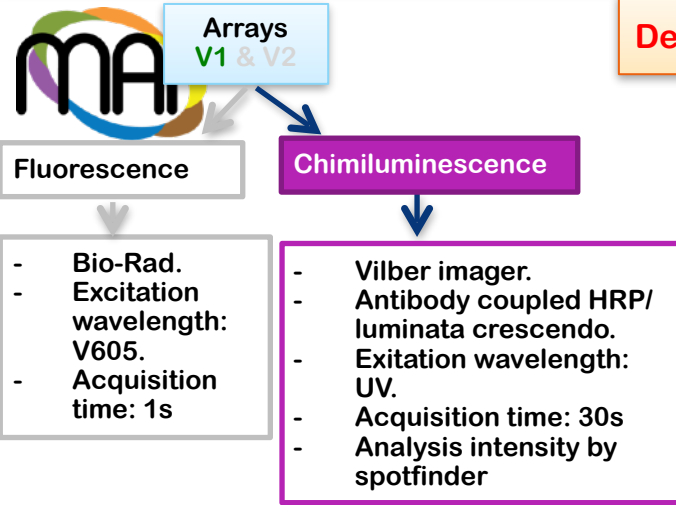
and

Chemiluminescence

- Vilber imager.
- Antibody anti-KdgM / Antibody coupled HRP / Luminata® Crescendo®.
- Excitation wavelength: UV
- Acquisition time: 30s
- Analysis intensity by **Spotfinder**

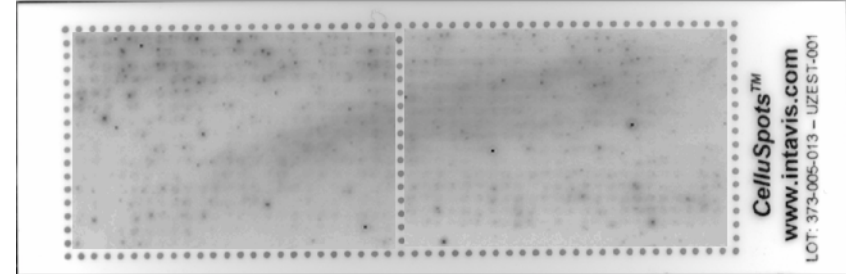
Fluorescence

- Bio-Rad imager.
- Excitation wavelength: 530 nm
- Emission wavelength: 605 nm
- Acquisition time: 1s
- Analysis intensity by **Image Lab**

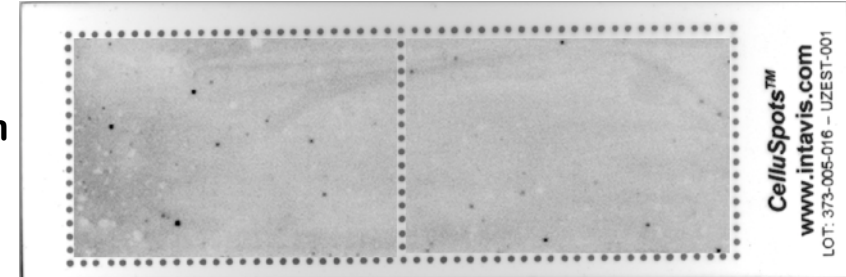


Detection by chemiluminescence (PepArray V1)

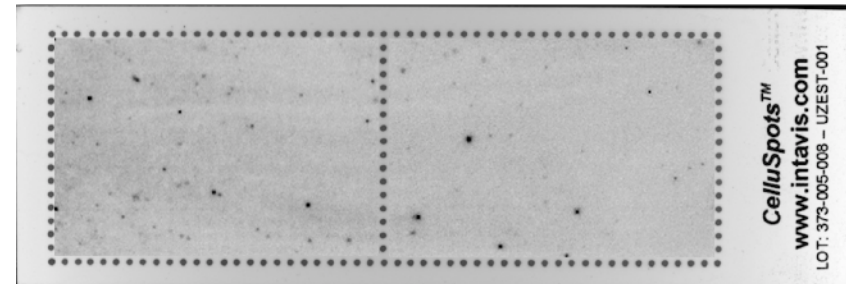
Dickeya dadantii



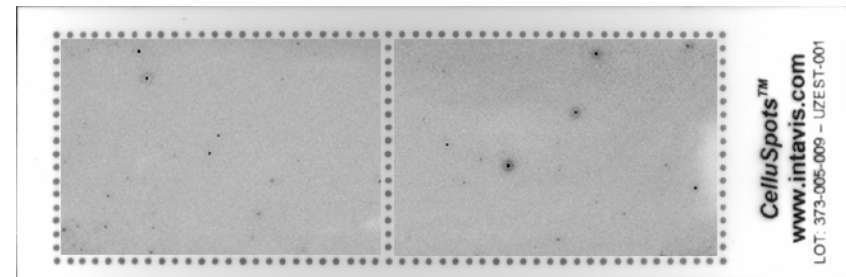
Agrobacterium tumefaciens

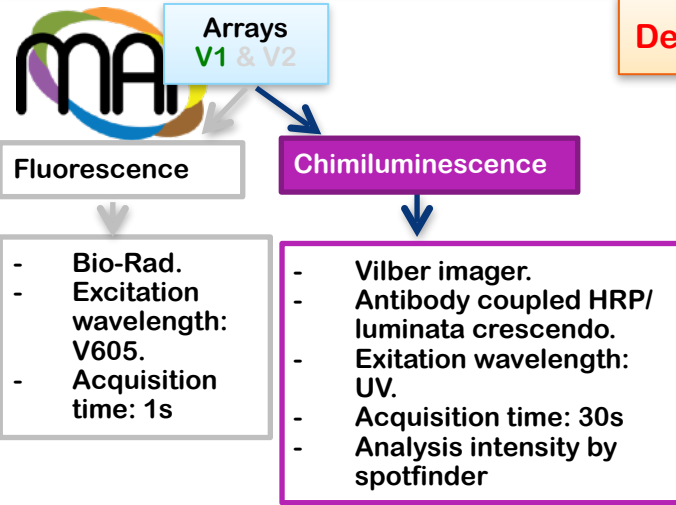


Erwinia aphidicola



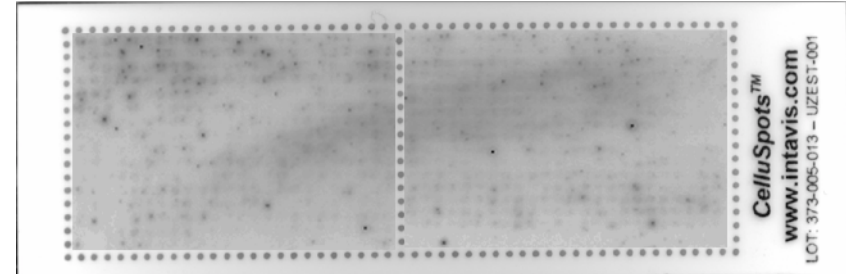
Pantoea agglomerans



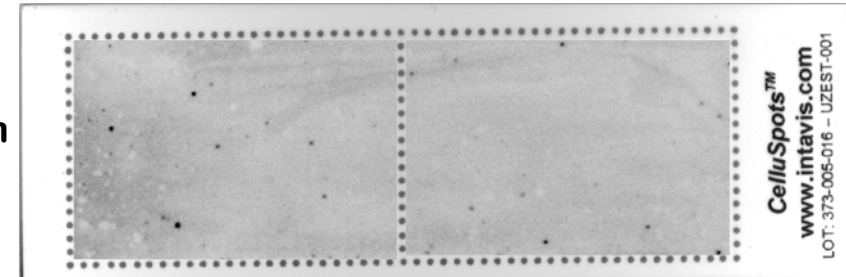


Detection by chemiluminescence (PepArray V1)

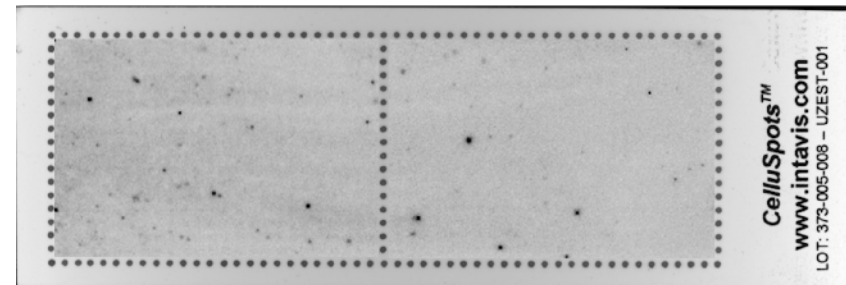
Dickeya dadantii



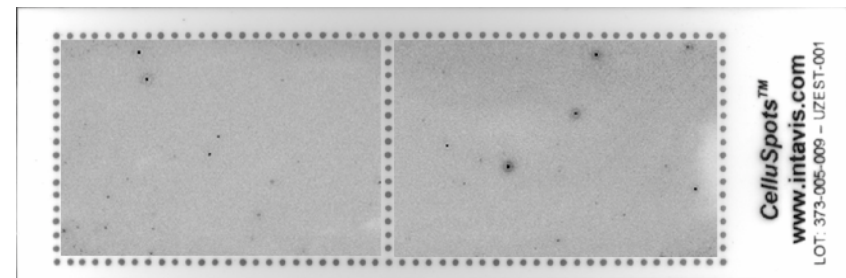
Agrobacterium tumefaciens



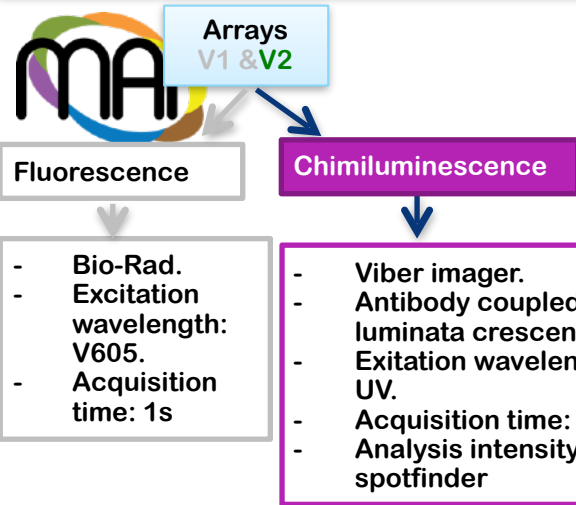
Erwinia aphidicola



Pantoea agglomerans

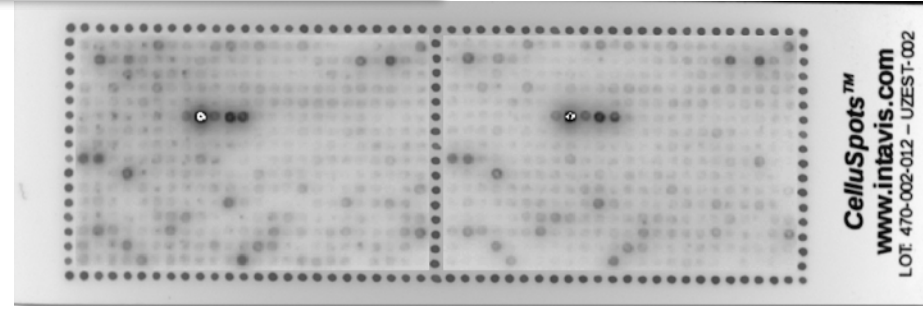


- Reproducibility issues
- Erratic spot issues
- No real fixation of the bacteria on V1 arrays ?

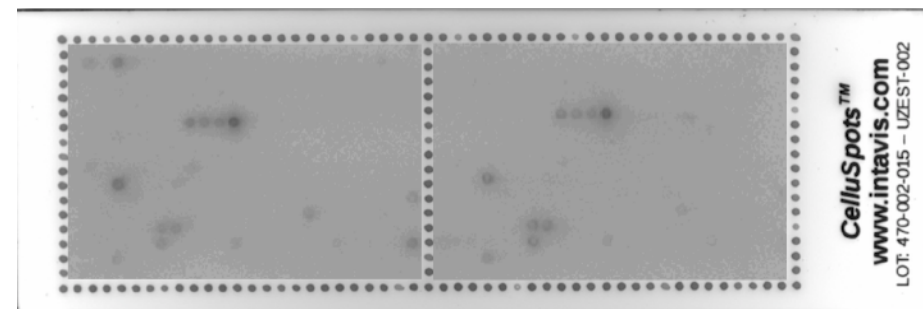


Detection by chemiluminescence (PepArray V2)

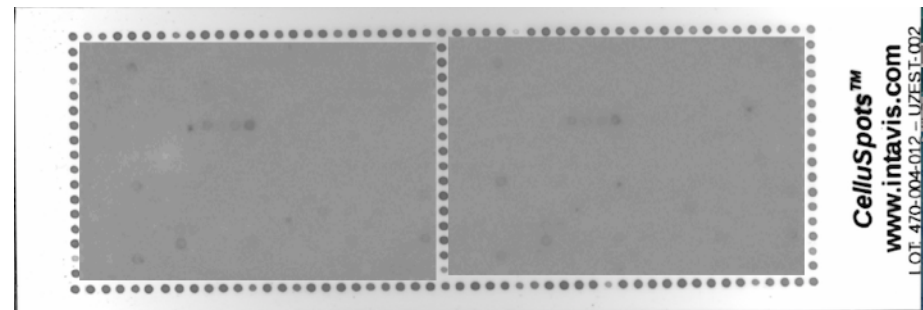
Dickeya dadantii



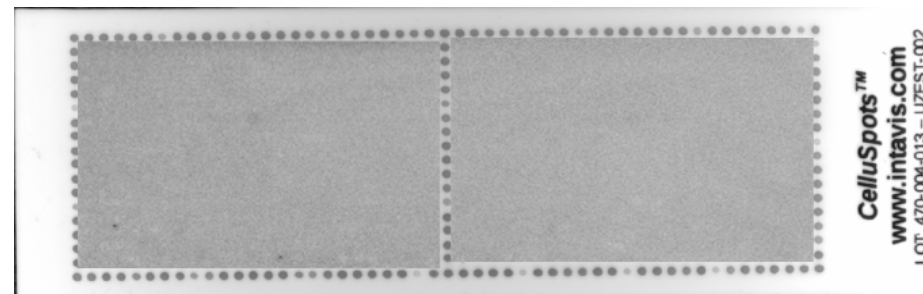
Agrobacterium tumefaciens

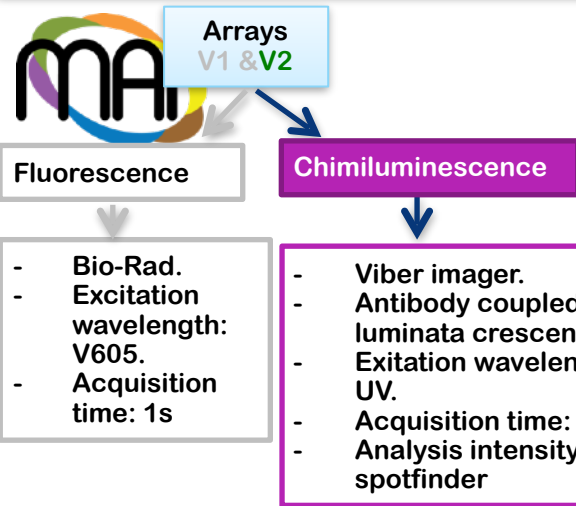


Erwinia aphidicola



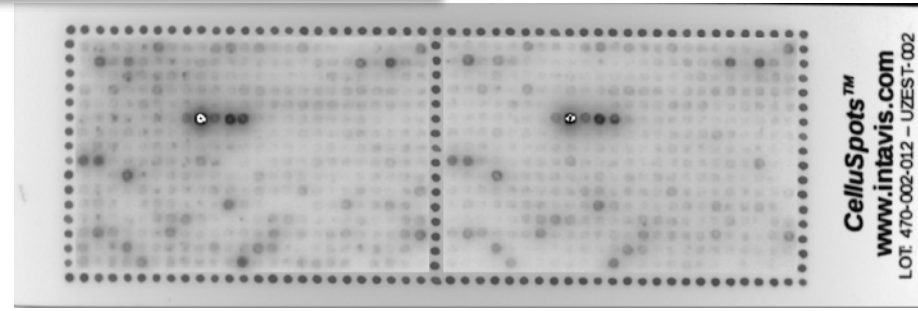
Pantoea agglomerans



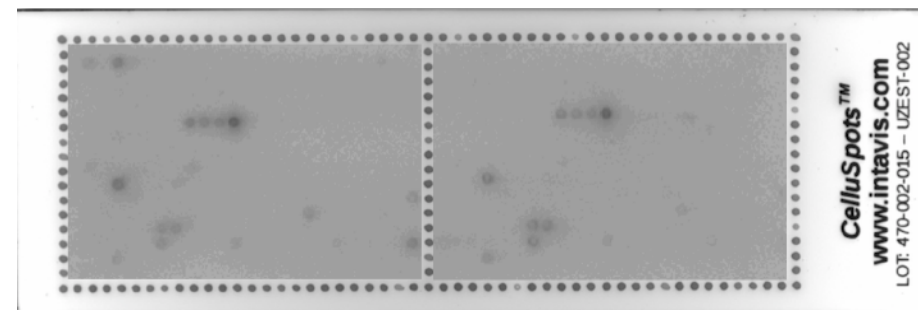


Detection by chemiluminescence (PepArray V2)

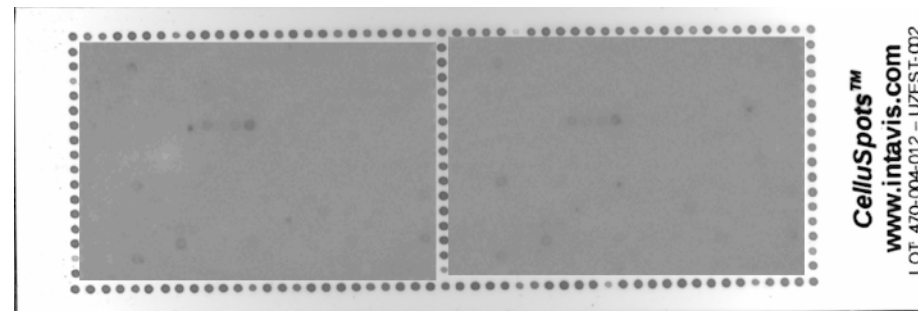
Dickeya dadantii



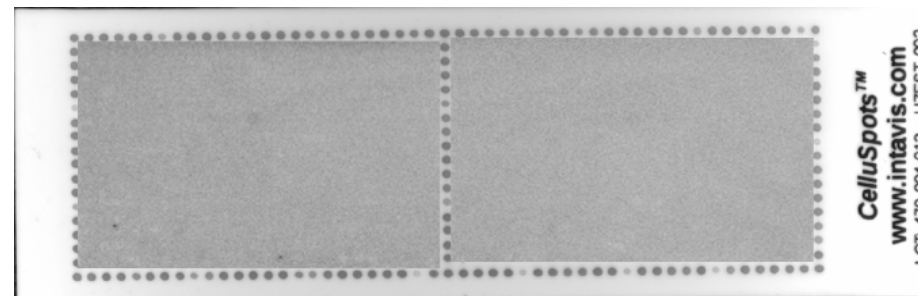
Agrobacterium tumefaciens



Erwinia aphidicola



Pantoea agglomerans



→ Spots detected on the arrays

→ Fixation of the bacteria on the peptides of the V2 arrays

→ Probable true positive signal, visible especially with *D. dadantii*



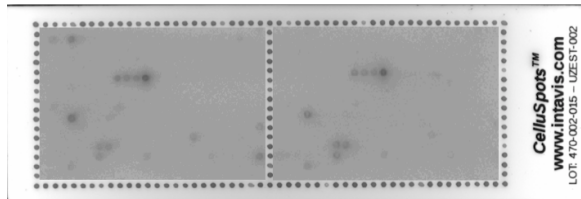
Analysis arrays (1)



Quantification by TIGR
spotfinder software

Computer (**excel**, **jmp**) analysis and
classification of signal intensities

Analysis arrays (1)

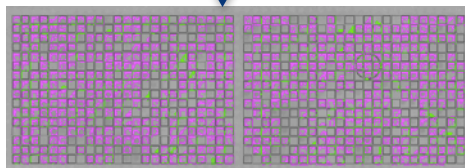
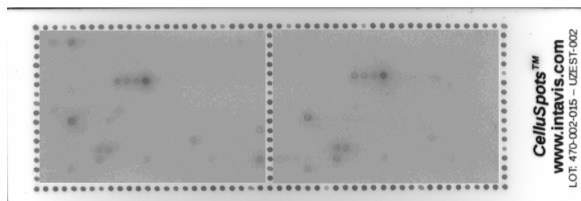



Quantification by TIGR
spotfinder software

Computer (**excel**, **jmp**) analysis and
classification of signal intensities



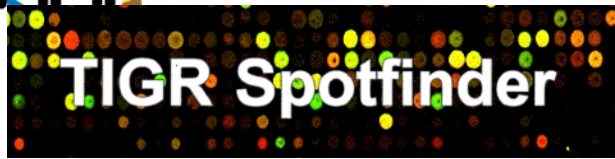
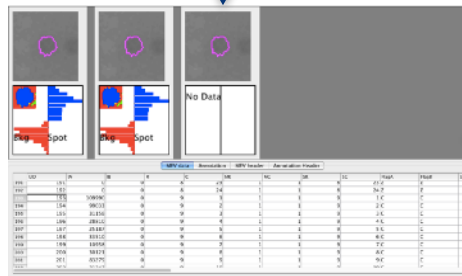
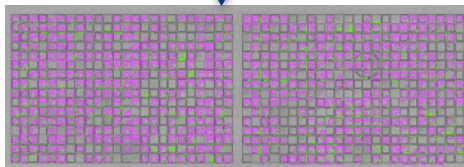
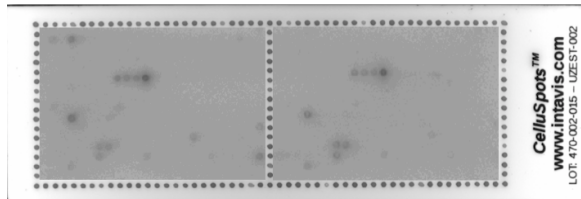
Analysis arrays (1)



Quantification by TIGR
spotfinder software

Computer (**excel**, **jmp**) analysis and
classification of signal intensities

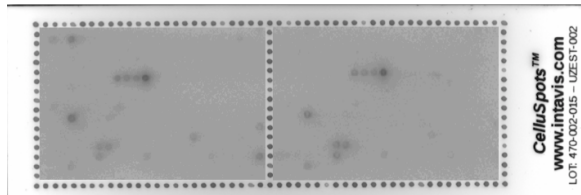
Analysis arrays (1)

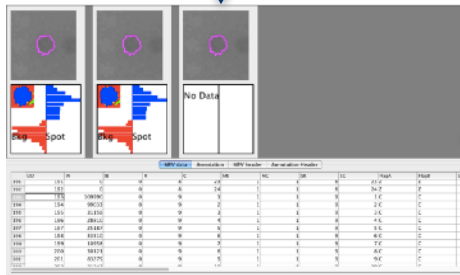
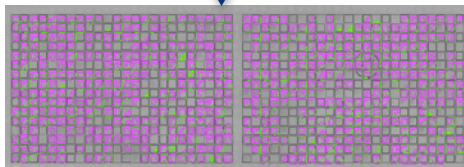
Quantification by TIGR
spotfinder software

Computer (**excel**, **jmp**) analysis and
classification of signal intensities

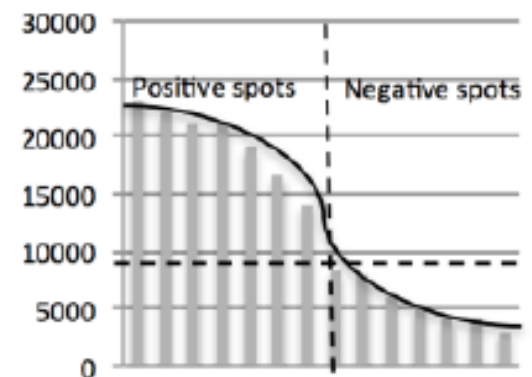
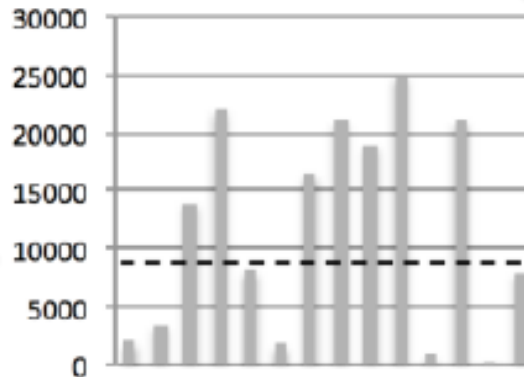
Analysis arrays (1)



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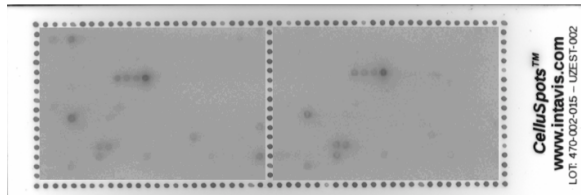


Quantification by TIGR spotfinder software

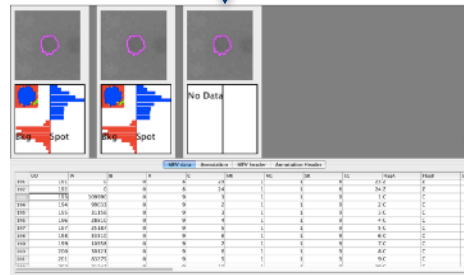
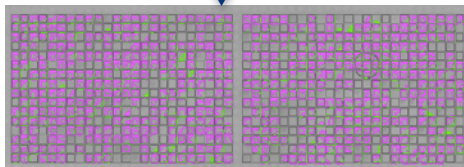


Computer (excel, jmp) analysis and classification of signal intensities

Analysis arrays (1)

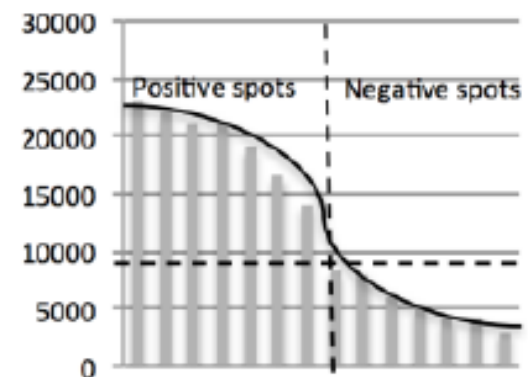
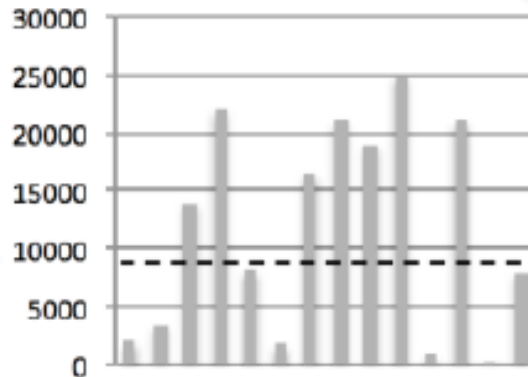


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Quantification by TIGR spotfinder software

Normalisation and subtraction by the negative controls

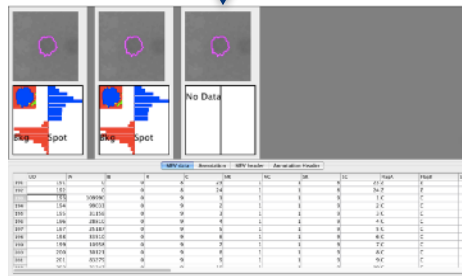
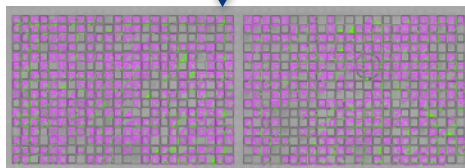
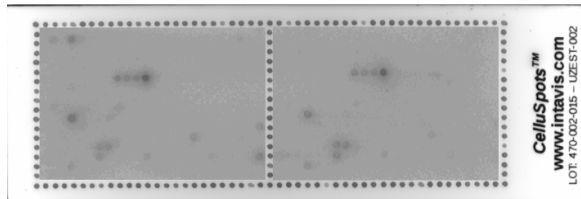


Computer (excel, jmp) analysis and classification of signal intensities

Analysis arrays (1)



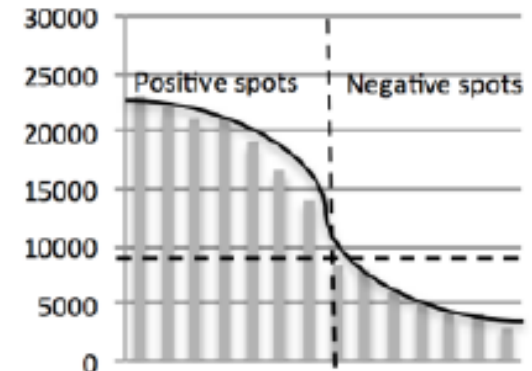
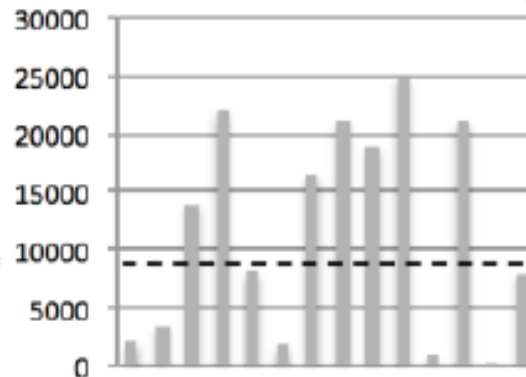
TIGR Spotfinder



Quantification by TIGR
spotfinder software

corresponding peptides (and protein / families) bind
bacteria, and are manually grouped by homology
groups are analysed (WebLogo)

Normalisation and subtraction
by the negative controls



Computer (**excel**, **jmp**) analysis and
classification of signal intensities



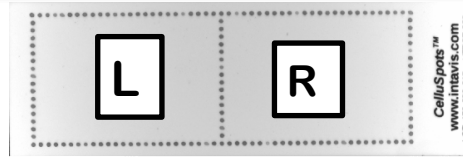
Analysis arrays (D.dadantii) (2)

B

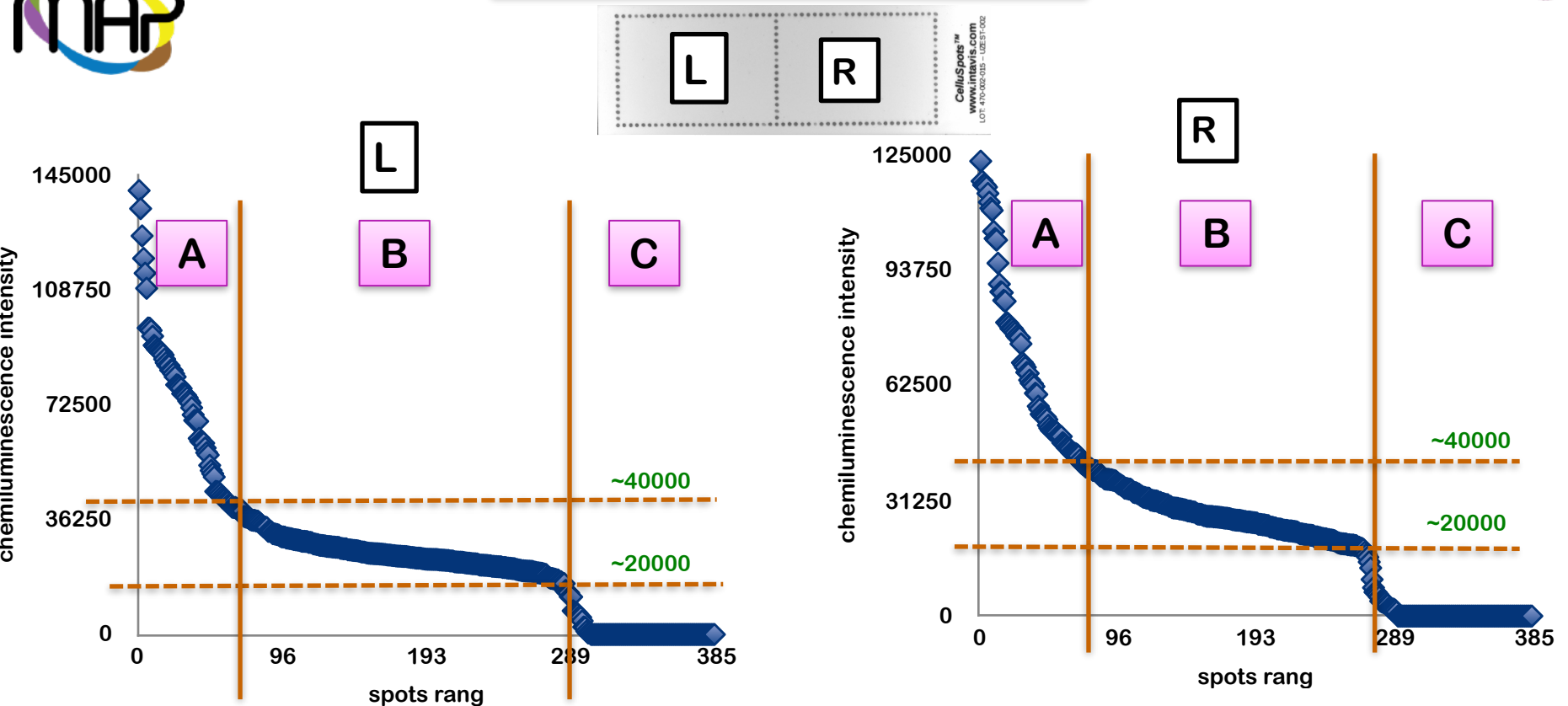
C



Analysis arrays (D.dadantii) (2)



Analysis arrays (D.dadantii) (2)



Chemiluminescence intensity spots of array incubated with *D.dadantii* (RFP) bacterias.

A: positive spots

B: negative spots

C: no signal detected

- 50 - 60 spots considered positive (intensity > 40 000).
- 180 – 200 spots considered negative (intensity ~40 000 – 20 000).
- 80 – 100 spots no detected (intensity < 20 000).

Analysis (D.dadantii) (2)

No de spot	Int-Moy Corr	seq peptidique	ACYPI	78	42,2		
326	97,8	YGFPGTAVPYGYGPTAVP	ACYPI010088	20	40,8	PTTPSSPSSSYQGNGFSY	ACYPI008113
293	94,4	DPYPAEDLRPNVGVKTQ	ACYPI004305	26	40,6	GVHPAIQRAVAQQVAQAK	ACYPI002877 & ACYPI000308
193	88,2	GSGYAFEFGGNNNGGAS	ACYPI006276	324	40,1	QNEFGQYSYGYNDGFSSK	ACYPI009491
308	85,5	PVRYAPSGSKPLKNTLAV	ACYPI008524	49	39,3	PVAEPSLYSAYLPPKPM	ACYPI010088
244	82,0	MTEPPTYFYQPVAKVAKR	ACYPI002781	142	38,0	DPLKQKQTLNSSPAKNS	ACYPI005591
349	78,9	RYADSSKSMSSRANNYYG	ACYPI44057	10	37,6	SLELIASQPQKPEDSKKK	ACYPI001775
227	78,1	QNAEDYNDQSAVVSARKS	ACYPI002781	337	36,4	SPYFNGFRPNGAYPQQYI	ACYPI002877 & ACYPI000308
350	75,2	YGVVQYAPVHVHTAAD	ACYPI44057	280	36,3	IEIIPASYAWSTFTGPVS	ACYPI38155
314	73,9	SYSAPSYSAPSYSAPSYS	ACYPI009152	24	35,7	QDNASPFNGTESNSIQRR	ACYPI001681
6	68,5	ENGYQPYGAHLPTPPPI	ACYPI009006	75	34,7	SLYFSGPQYYSGYAAPA	ACYPI009491
194	68,2	YAFEFGGNNNGGASPEY	ACYPI006276	8	34,5	SIEPQOMWYVNNQQGRY	ACYPI005591
245	62,8	AKRTRPAAAQLSTNYRTA	ACYPI002781	304	33,6	ESLRYLASLPSTPEPKYQ	ACYPI009006
267	62,5	APAGQHHTVVPILKQVNR	ACYPI000890	83	33,6	EAKVVATAPAKIVTPTRT	ACYPI008524
205	59,7	SDGGKSAAVGVKKVLVSK	ACYPI37842	315	32,9	QSGVSSYQSTPASLQYSK	ACYPI008113
203	59,6	DPTAASVVQTTGRRNKK	ACYPI37842	271	32,4	SSYSSSSYSSSSYSTAAP	ACYPI009152
361	59,3	RRPSPVNDVSVQRRQQQ	ACYPI51165	102	32,4	APVAVRQQQWFSAGPQQK	ACYPI000890
184	58,2	APAYVSPSPSGRSLAKNR	ACYPI006276	374	32,2	DGSYSYSYETGNGISVSE	ACYPI008113
224	53,9	LSTSPRYDPRISERSTT	ACYPI37842	52	31,2	KTISKARGQPLEPKVYTM	Pep-M2.2
28	53,3	AYSYVDPNGALQQYKYVS	ACYPI009491	77	30,9	YNFKYETGNGIFAERGV	ACYPI005591
11	53,2	QQYIGGPQQQFIGGPNFA	ACYPI002877	234	30,7	HPQGYESEASSGYGSPTT	ACYPI008113
21	51,1	AVAQQVAQAKLEPPQFNK	ACYPI002877 & ACYPI000308	325	30,4	DEEPAAPVPVKRPSPAKP	ACYPI002781
201	50,6	DVGRCLKRVSYASNSSGF	ACYPI37842	309	30,3	KPMVVVGGPLHYNSPYDG	ACYPI010088
163	47,3	GSYSYVDPNGQRRTVSYT	ACYPI006250	229	30,1	KPLKNTLAVPETKTKTGY	ACYPI008524
135	44,0	PVYKNVDNKLPAIVRQSQ	ACYPI001775	148	30,1	HNEDGSYTYGYEGSDGSF	ACYPI002781
365	42,5	NLNDGRITTLGDKQPSV	ACYPI51165			YQVEYFADADNGFVAKGA	ACYPI066082

→ 50 peptide sequences correspond to 50 positive spots

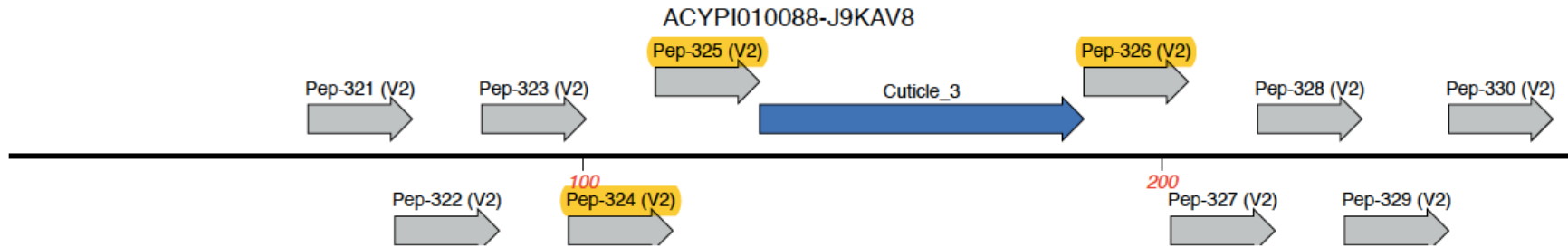
→ belong to 23 CuPs

→ For example: 3 peptides sequences (326, 324, 325) from CuP ACYPI010088



Analysis (*D.dadantii*) (3)

For example



3 peptide sequences (pep-324, pep-325 and pep-326)
of CuP ACYPI010088 (CPR unclassified)



Conclusion

- Interaction of *D.dadantii* with some CuPs proteins from CRP family was detected.
- No interaction of *D.dadantii* bacterias with the major CuP RR2 subfamily.
- 23 CuPs (RR1 and Unclassified) are able to take part in the interaction with *D.dadantii* in vitro.



Perspectives

- Test a colorimetric method
 - More reproducible ?
 - Less sensitive ?
 - Non re-usable
- SEM of hybridized arrays
- Non Dickeya : change to other primary antibody
- Test purified major bacterial envelope components:
 - Extract pilus and flagellum and hybridize (V1 ?, V2)
- Incubation with mutant bacteria to test outer membrane components (kdgM, ompA, ompF ...)
- Impact of growth phase on binding
- Go to in vivo situation in aphid anterior digestive tract (SEM of dissected stylets - oesophagum). Entry OK, Binding ?