

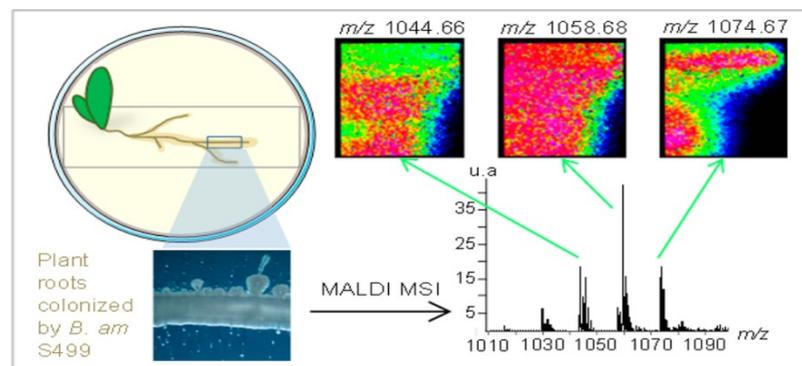
Spatiotemporal Monitoring of the Antibiofilm Secreted by *Bacillus* Biofilms on Plant Roots Using MALDI Mass Spectrometry Imaging

Delphine Debois,^{*,†,||} Emmanuel Jourdan,^{‡,||} Nicolas Smargiasso,[†] Philippe Thonart,[§] Edwin De Pauw,[†] and Marc Ongena^{*,§}

[†]Mass Spectrometry Laboratory, Chemistry Department, University of Liege, 4000 Liege, Belgium

[‡]Centre Wallon de Biologie Industrielle, University of Liege, 4000 Liege, Belgium

[§]Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium

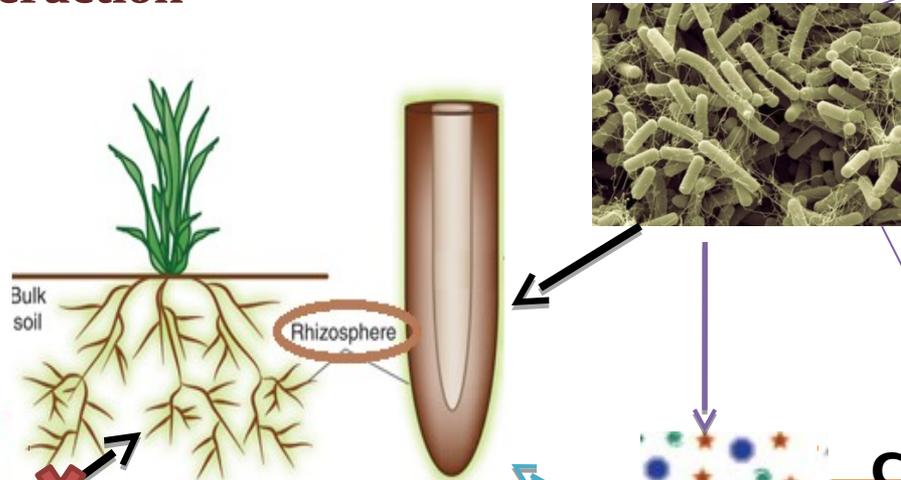


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Introduction

Tritrophic Interaction

Bacillus amyloliquefaciens



• More than 8% of the genetic equipment dedicated to nonribosomal synthesis of lipopeptide (LP)- and polyketide (PK)-type antibiotics.

Challenge: Inherent difficulties in detecting these small size compounds in complex matrices

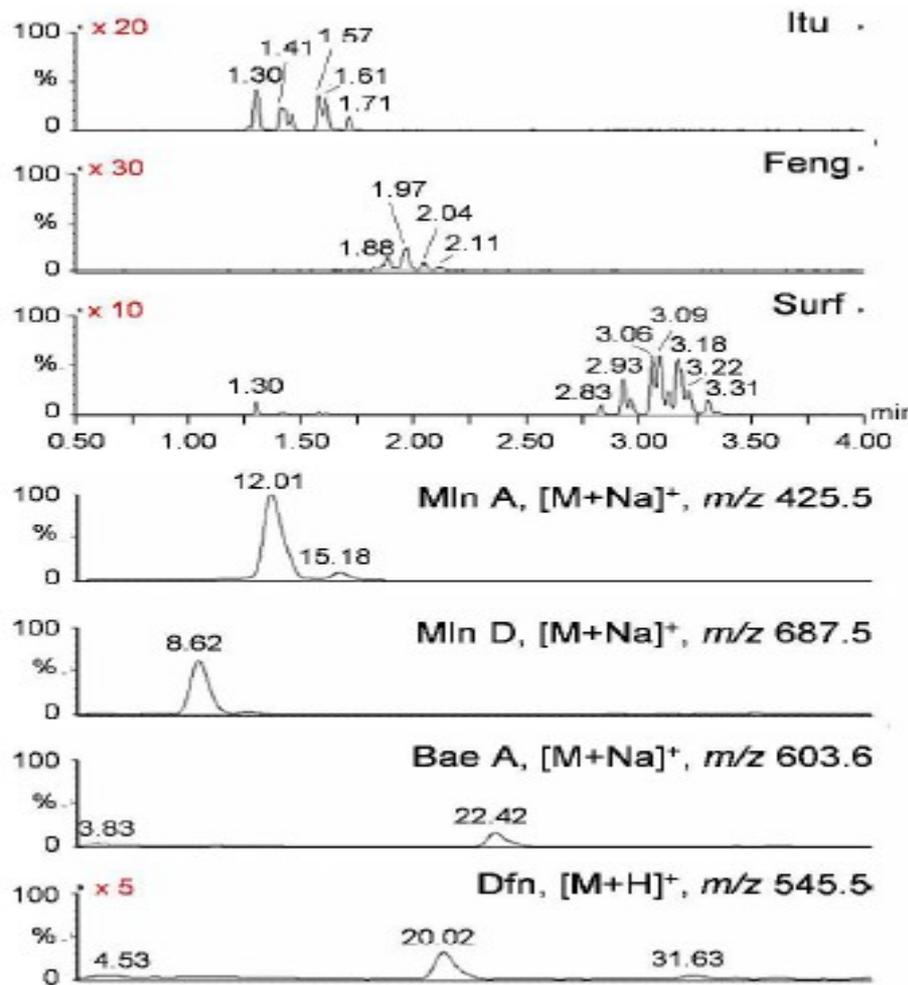
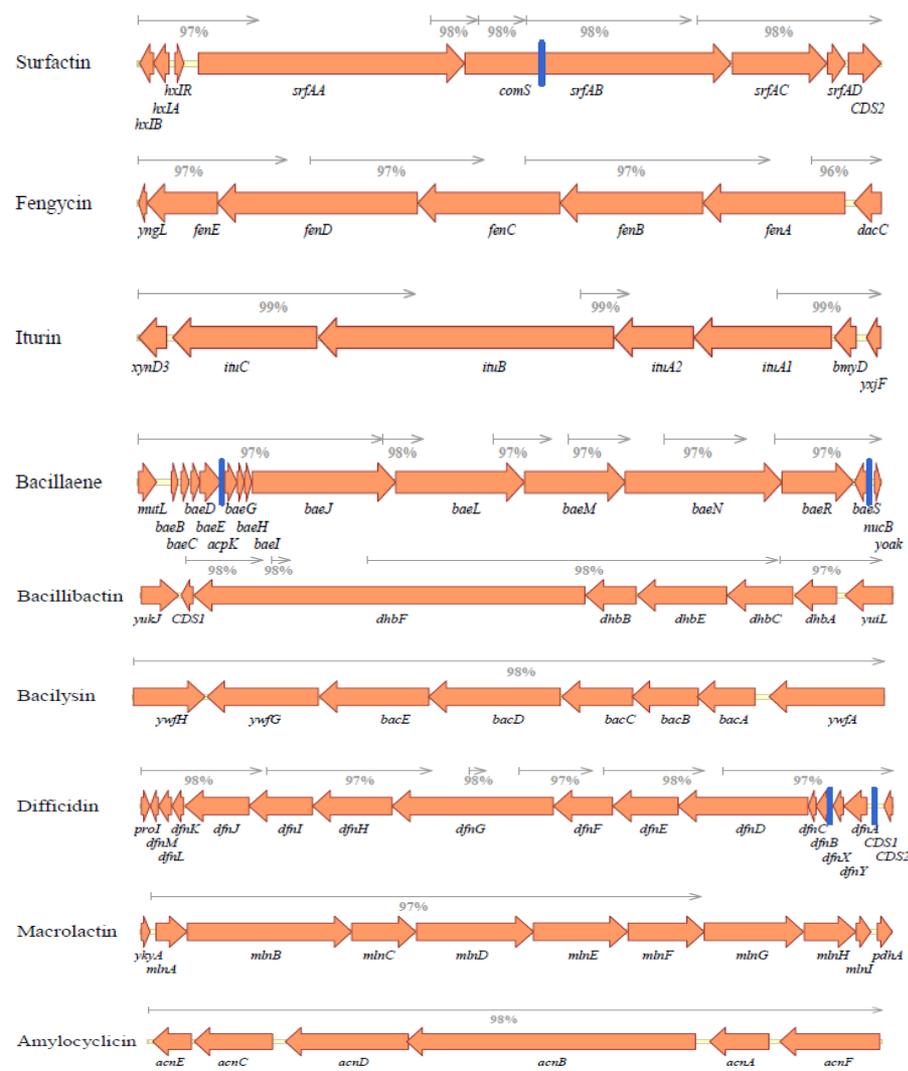
Other organisms in the same ecosystem

Antibiome

In this Study

- To provide insights into the variety of antibiotics that can be secreted by *Bacillus* cells evolving on plant roots.
- Exploit MALDI MSI to monitor the spatiotemporal accumulation of NRPS/PKS compounds produced in the rhizosphere by the plant-beneficial *B. amyloliquefaciens* strain S499.

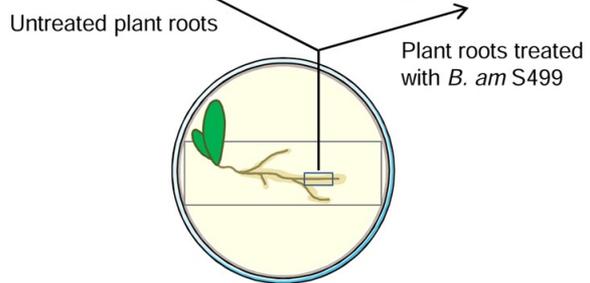
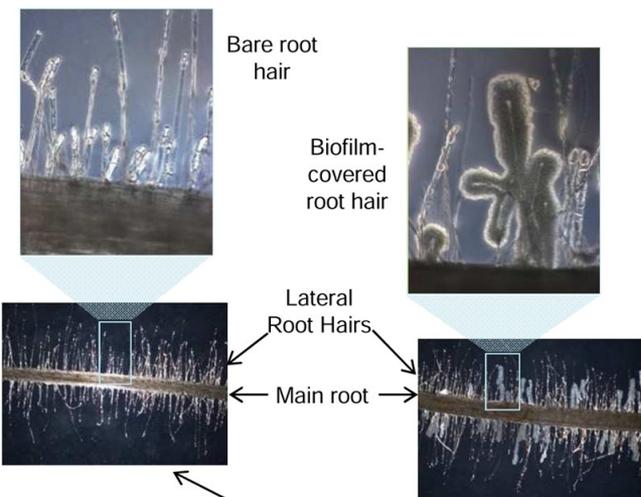
Potential of *B. amyloliquefaciens* S499 for Production of NRPS/PKS Antibiotics



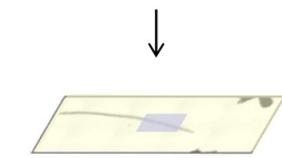
Overview of NRPS and PKS antibiotic gene fragment identified in the genome of *Bacillus*

Extracted ion chromatogram using UPLC-ESI-MS for surfactins, iturins, fengycins, marcrolactin A, macrolactin D, baecillaene A, and

MS Imaging of the S499 Antibiofilm Produced on Different Plants

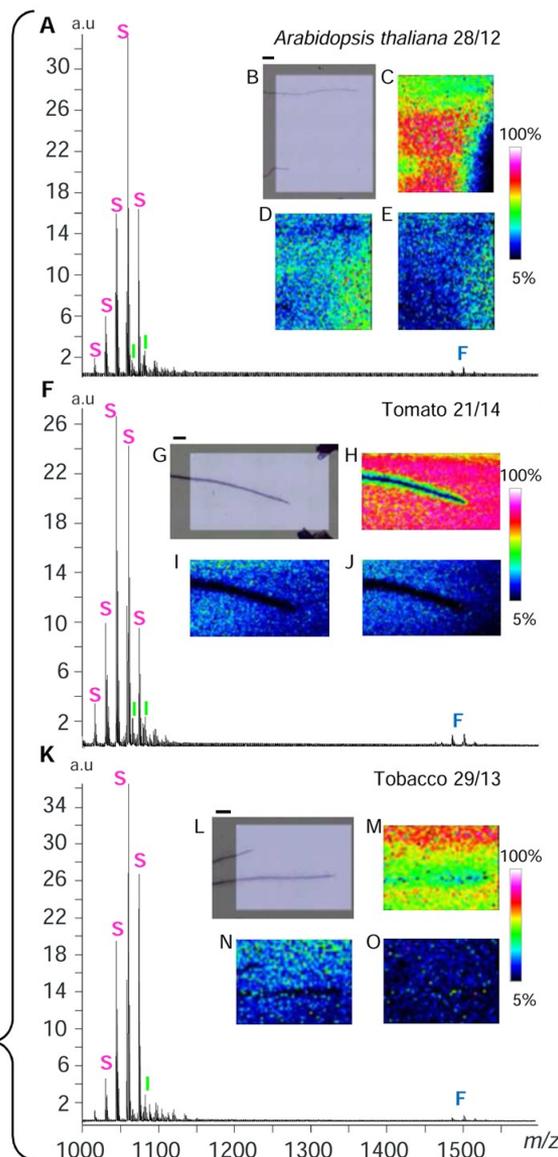


Leaves and roots are cut to allow the ITO-coated glass slide to be removed from the semi-solid agar medium and dried under reduced pressure during 1h30-2h



Matrix is deposited using an automated sprayer

The sample is ready for acquisition



(A) Average MALDI mass spectrum recorded on an *A. thaliana* root aged of 28 days, incubated with *B. amyloliquefaciens* S499 during 12 days

(B) Microscope picture of the analyzed area on the root

(C, D, E) MALDI image of $[C14 - Surf + Na]^+$ ion, $[C14 - ItuA + Na]^+$ ion, $[C14 - FengB + Na]^+$ ion, respectively.

(F) average MALDI mass spectrum recorded on tomato root aged of 21 days, incubated with *B. amyloliquefaciens* S499 during 14 days

(G) Microscope picture of the analyzed area on the root

(H, I, J) MALDI image of $[C14 - Surf + Na]^+$ ion, $[C14 - ItuA + Na]^+$ ion, $[C14 - FengB + Na]^+$ ion, respectively.

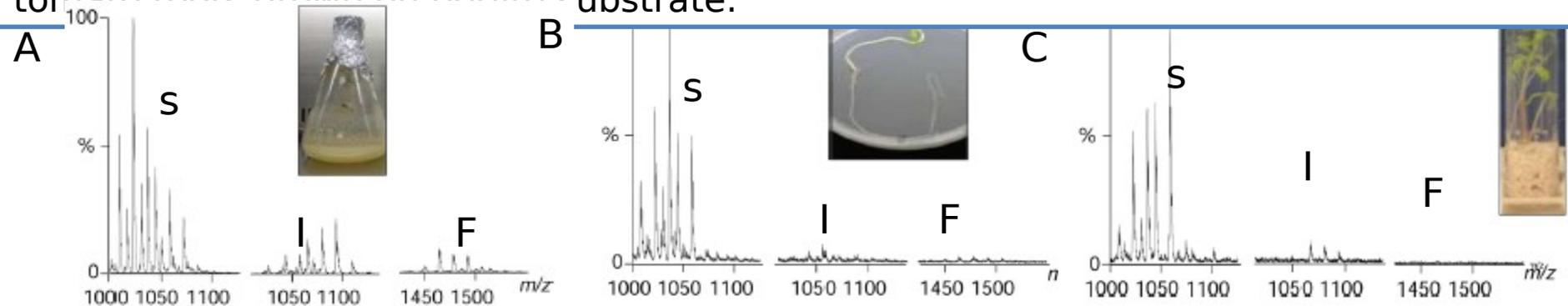
(K) Average MALDI mass spectrum recorded on tobacco root aged of 29 days, incubated with *B. amyloliquefaciens* S499 during 13 days

(L) Microscope picture of the analyzed area on the root

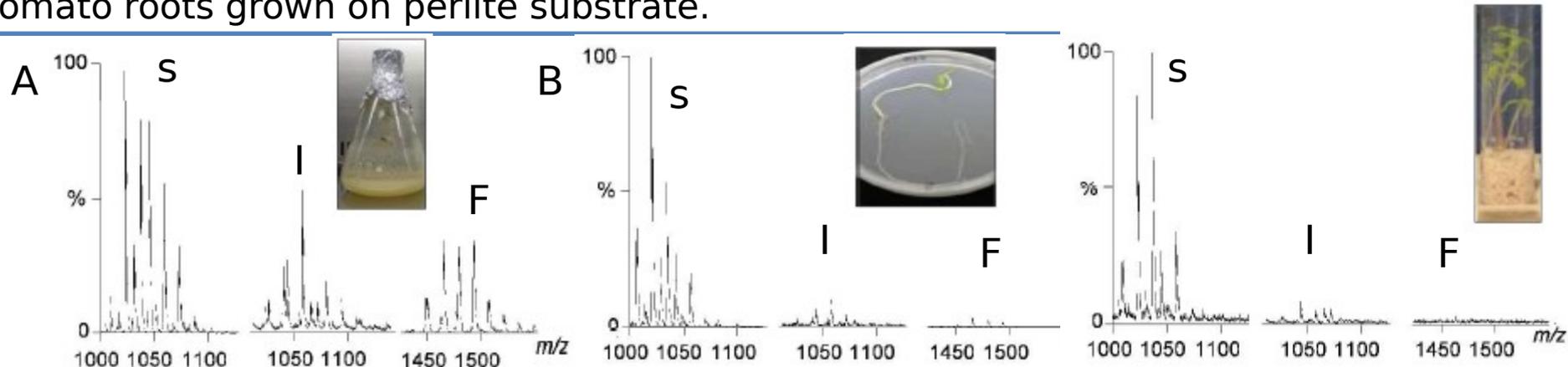
(M, N, O) MALDI image of $[C14 - Surf + Na]^+$ ion, $[C14 - ItuA + Na]^+$ ion, $[C14 - FengB + Na]^+$ ion, respectively.

Strain and Culture Medium Independent Antibioime Profile

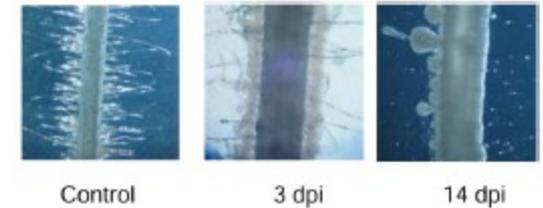
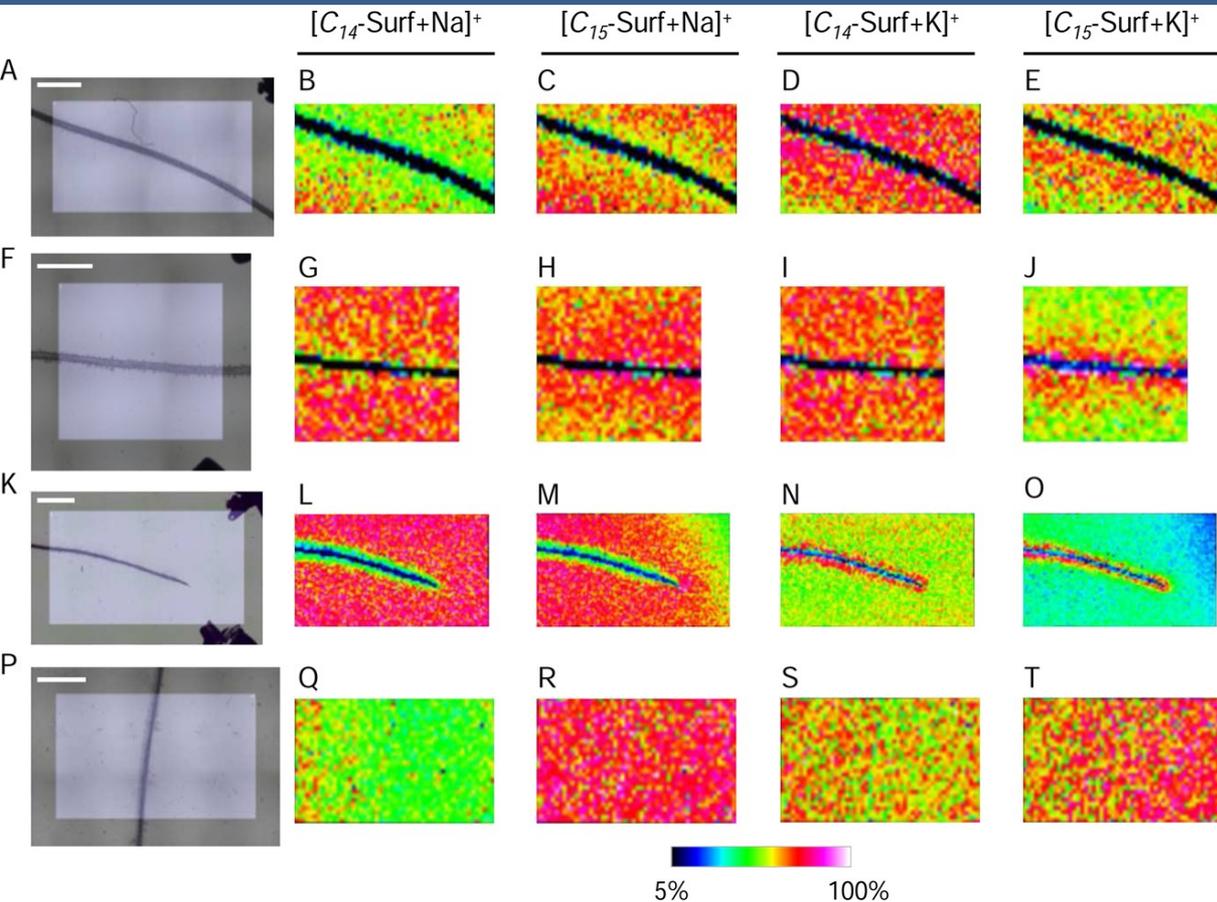
Extracted ion chromatograms of each LP family from extracts prepared from different cultivations of *B. amyloliquefaciens* **S499**: (A) supernatant of liquid cultures, (B) surface of the gelified medium surrounding tomato roots, and (C) root environment of tomato roots grown on perlite substrate.



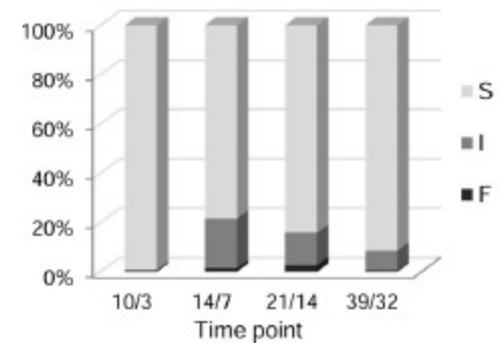
Extracted ion chromatograms of each LP family from extracts prepared from different culture modes of *B. amyloliquefaciens* **GA1**. (A) supernatant of liquid cultures, (B) surface of the gelified medium surrounding tomato roots, (C) root environment of tomato roots grown on perlite substrate.



Time-Course Imaging of LPs Pattern on Tomato Roots



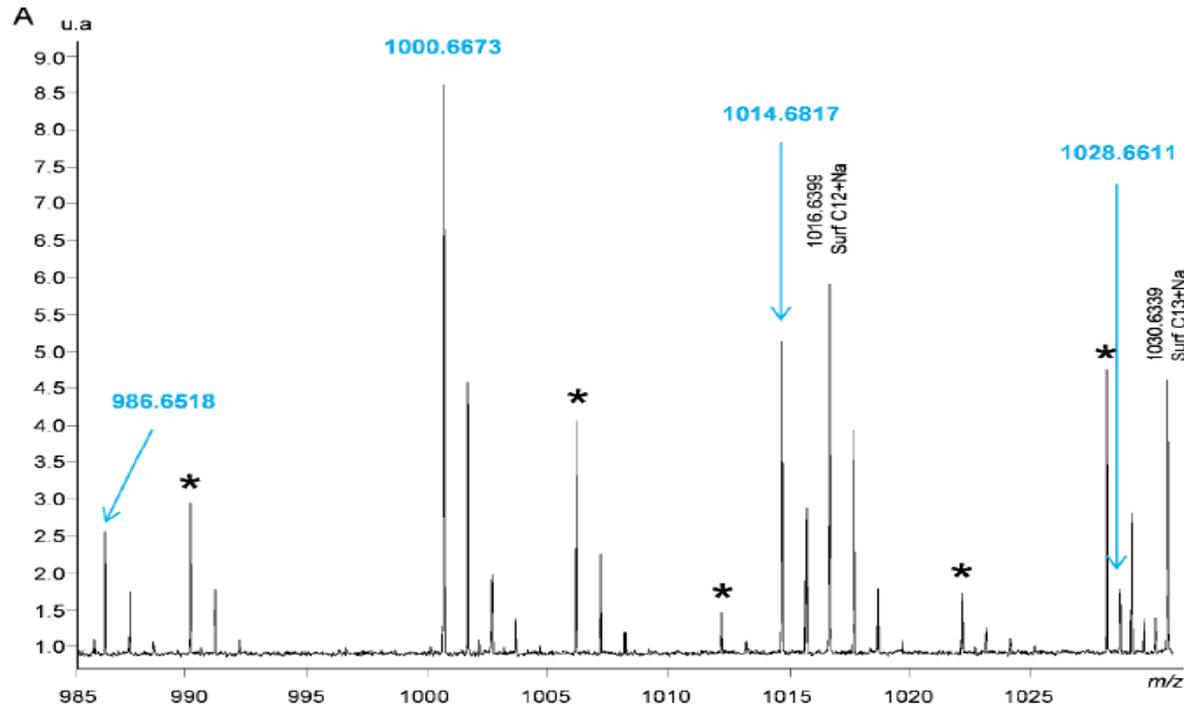
Pictures of biofilms formed by strain S499 on tomato roots at 3 and 14 dpi.



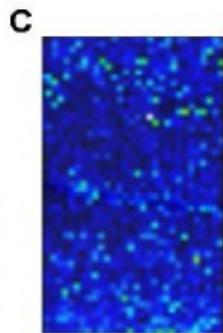
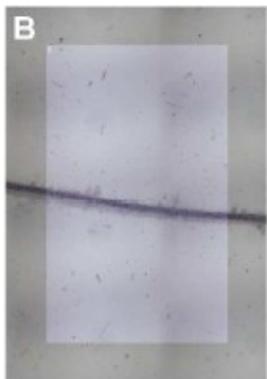
Relative abundances of the secreted LPs families over time

(A, F, K, P) Microscope images of tomato roots colonized by S499 for 3, 7, 14, and 32 days, respectively. (B, G, L, Q) MALDI images of $[M + Na]^+$ of C14 – surfactin (m/z 1044.66). (C, H, M, R) MALDI images of $[M + Na]^+$ of C15 – surfactin (m/z 1058.67). (D, I, N, S) MALDI images of $[M + K]^+$ of C14 – surfactin (m/z 1060.64). (E, J, O, T) MALDI images of $[M + K]^+$ of C15 – surfactin (m/z 1062.66).

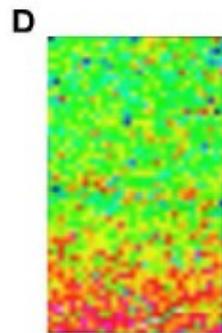
Detection and Structural Analysis of a New Variant of Surfactin



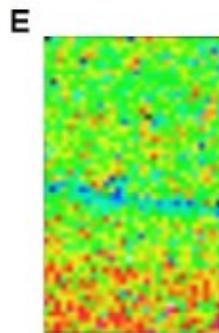
(A) MALDI FTICR mass spectrum recorded on a tomato root colonized by *B. amyloliquefaciens* S499 for 32 days, showing the exact masses of new variants of surfactins. Stars indicate MALDI matrix adducts peaks. (B) microscope picture of the sample, the grey square defines the analyzed area. (C) MALDI-TOF image of ion at various m/z values.



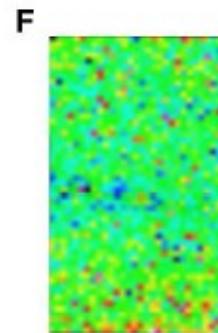
m/z 986.66



m/z 1000.68

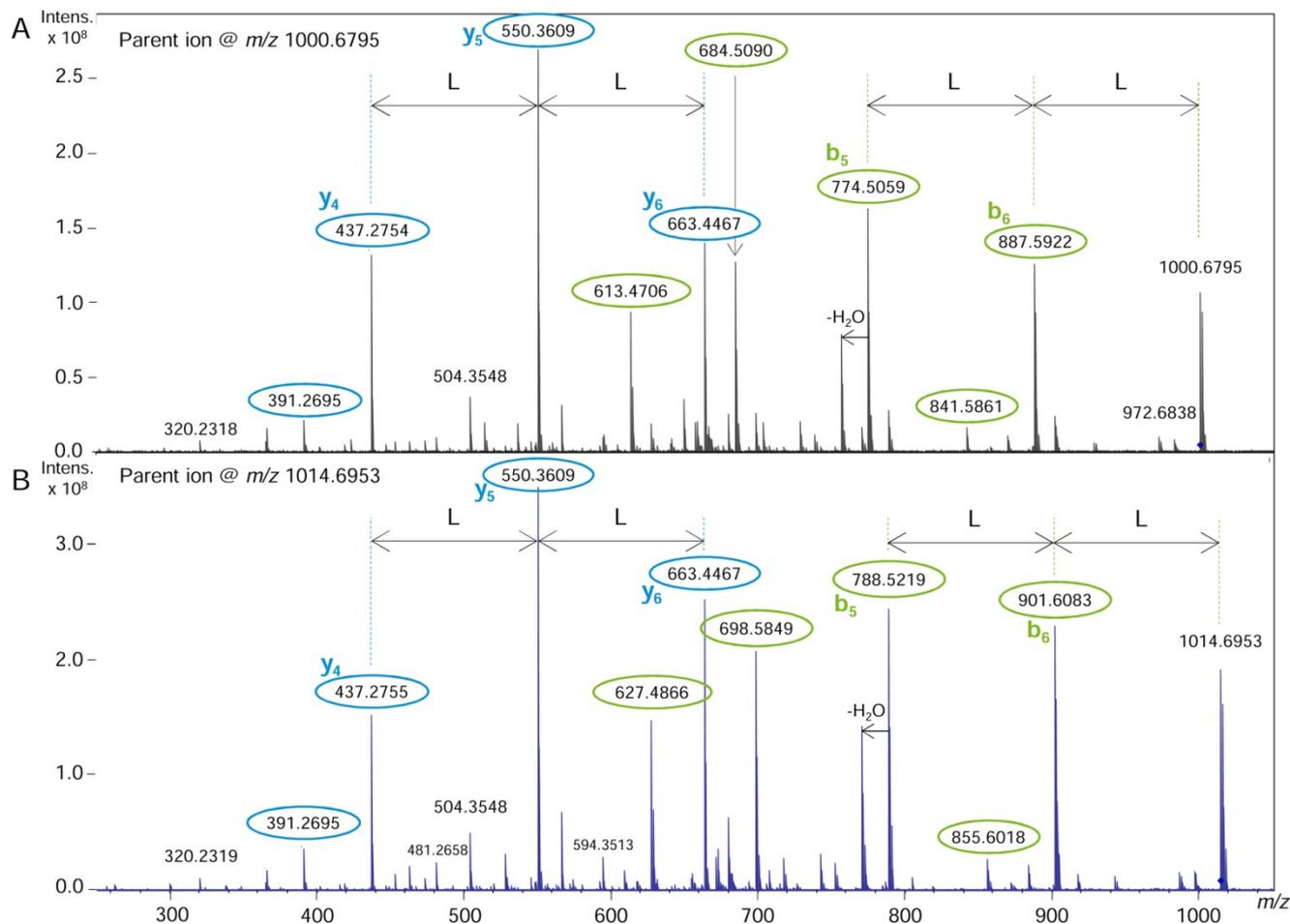


m/z 1014.69



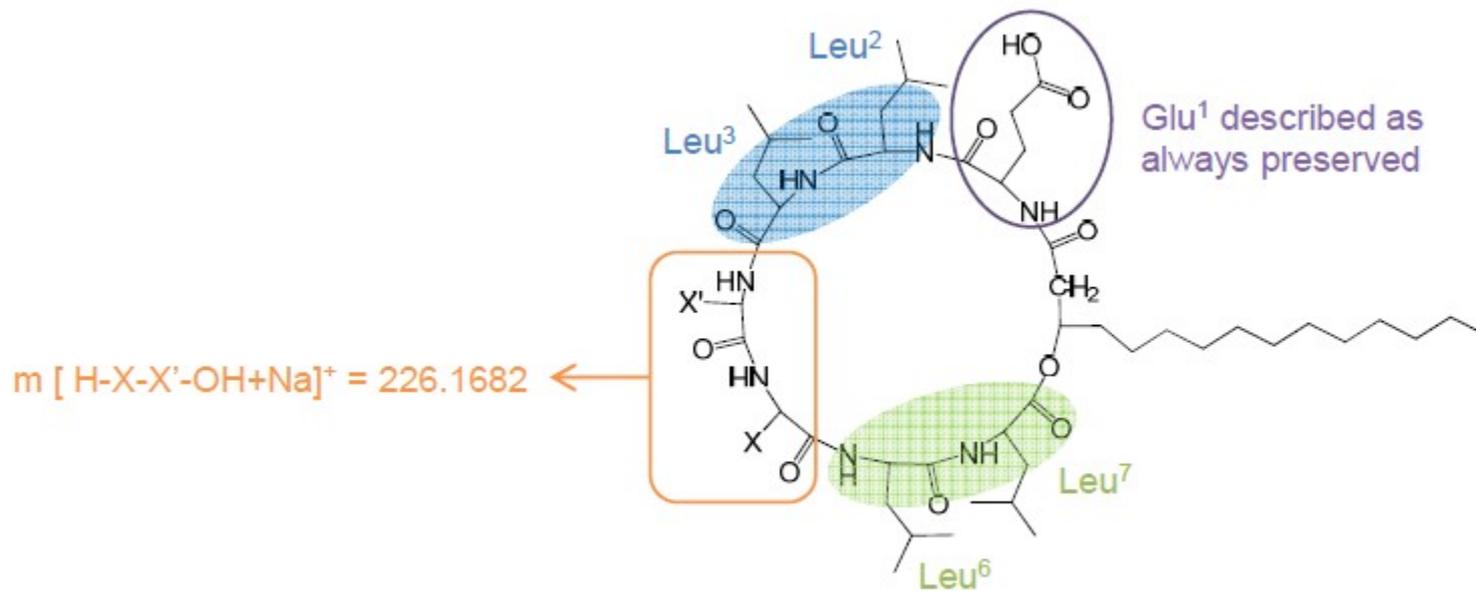
m/z 1028.68

Detection and Structural Analysis of a New Variant of Surfactin



(A and B) MS/MS spectra recorded on the environment of a 39 days old tomato root, 32 dpi with S499 cells, with m/z 1000.6795 and m/z 1014.6953 as precursor ion, respectively.

Chemical Structure of Unknown Surfactin



Summary

- MS imaging has revealed as powerful tool to identify antibiotic compounds released by root colonizing *Bacillus* cells and to spatiotemporally resolve their dynamics of production.
- Globally, data strongly suggest that the antibiome expressed in planta by *B. amyloliquefaciens* isolates does not reflect the vast genetic arsenal devoted to the formation of bioactive compounds by these bacteria.
- Some of these *Bacillus* antibiotics may reach threshold concentrations for activity within the rhizosphere, while others would remain below this level or simply not produced.
- This new methodology can be obviously adapted for monitoring spatiotemporal antibiotic production patterns from other bacterial genera/species of interest as biocontrol agents.
- MSI could also be exploited as tool for selecting isolates particularly efficient at expressing their antibiome in planta among a first set of strains primarily screened for huge antibiotic potential in high-throughput and well-defined in vitro conditions.

Thank you