

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

From Research  
From Lab

...to Market  
...to Field

USJ 1875  
Université Saint-Joseph de Beyrouth  
جامعة القديس يوسف في بيروت

Faculté des sciences  
Université Saint-Joseph de Beyrouth

ATOBT

ASSOCIATION  
TUNISIENNE DE BIOTECHNOLOGIE  
IDB2022 – 20<sup>th</sup> December

**WP2-3. Proof of concept, from *B. thuringiensis kurstaki* cultivation to  $\delta$ -endotoxin production (bioperformances, bioprocess and scale-up)**

**In-depth genomic analysis of *Bacillus thuringiensis* serovar *kurstaki* strains: Genome dynamics in play.**

*Nancy FAYAD, Rita BARSSOUM, Nathalie MARSAUD, Julien CESCUT, Luc FILLAUDEAU, Mireille KALLASSY*

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

From Research  
From Lab

...to Market  
...to Field

USJ 1875  
Université Saint-Joseph de Beyrouth  
جامعة القديس يوسف في بيروت

Faculté des sciences  
Université Saint-Joseph de Beyrouth

ATOBT

ASSOCIATION  
TUNISIENNE DE BIOTECHNOLOGIE  
IDB2022 – 20<sup>th</sup> December

*Stepping in to the world of *Bacillus thuringiensis* genomics*

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

2

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

**Introduction – *B.c.s.l.***

From Research From Lab ...to Market ...to Field

Phylogenetically close

*Bacillus cereus sensu lato*

Ecologically diverse

Genomically rich

ANTHRAX

BIOPESTICIDES

Logos: INSA, tbi, INRA, CFA, USJ, etc.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

3

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

**Introduction – *B.c.s.l.***

From Research From Lab ...to Market ...to Field

- Phylogenetic analysis to distinguish between *Bacillus cereus sensu lato* members:
  - Phenotypic observations
  - 16S rDNA sequencing
  - Housekeeping genes
  - Whole genome sequencing (WGS)**

DX SIN

Logos: INSA, tbi, INRA, CFA, USJ, etc.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

4

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

From Research From Lab ...to Market ...to Field

### Introduction – WGS in *B.c.s.l.*

The diagram shows the hierarchical assembly of genomic data. It starts with 'Reads' (short blue horizontal bars), which are linked together to form 'Contigs' (longer blue bars). These contigs are further ordered and oriented to create 'Scaffolds' (red bars with gaps). Finally, all scaffolds are joined to form the complete 'Genome' (a long grey bar), which is highlighted with an orange border.

Logos: INSA, tbi, INRA, CFA, USJ, etc.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

5

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

From Research From Lab ...to Market ...to Field

### Introduction – WGS in *B.c.s.l.*

The diagram consists of three concentric orange circles. The innermost circle represents 4 genomes, the middle circle represents 79 genomes, and the outermost circle represents 429 genomes. Lines connect these circles to their respective text descriptions.

- 4 complete genomes reported for *Bacillus thuringiensis* serovar *kurstaki* Btk: HD73, YBT1520, BMB1 & **HD-1**
- 79 complete genomes reported for *Bacillus thuringiensis*
- 429 complete genomes reported in the *Bacillus cereus sensu lato* group


**Why re-sequence HD-1?**

Logos: INSA, tbi, INRA, CFA, USJ, etc.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

6

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

 **Introduction – WGS in *B.c.s.l.***


From Research From Lab ...to Market ...to Field

A ~ 5.5 Mb chromosome

Variable number: 1 – 14 plasmids

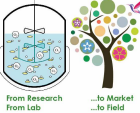
Sizes ranging between 2 – 600 kb

Important genomic rearrangements :  
Mobile Genetic Elements




This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921. 7

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

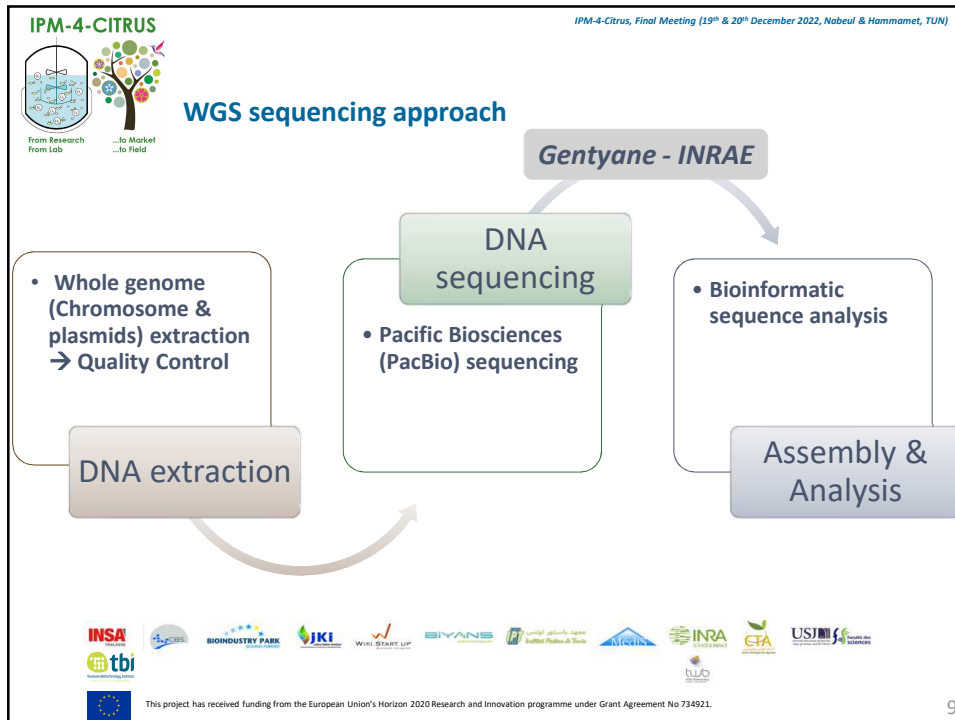
 **This study...**

From Research From Lab ...to Market ...to Field

- ... fits within the scope of the WP2
  - Sequence the three star strains:
    - *Btk* Lip: isolated from Lebanon
    - *Btk* BLB1: isolated from Tunisia
    - *Btk* HD-1: the reference strain for anti-lepidopteran activity
  - Highlight their various genomic features
  - Mine the  $\delta$ -endotoxin genes



This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921. 8



**IPM-4-CITRUS** IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

**Results: General WGS data**

Based on previous sequencing data....


	<b>HD-1</b>	<b>BLB1</b>	<b>Lip</b>
<b>Genome completeness (BUSCO v.5)</b>	99.5%	99.69%	99.78%
<b>GC percentage (%)</b>	34.89%	34.94%	34.93%
<b>Number of plasmids post assembly &amp; verification</b>	13	10 ??	11 ??
<b><i>kurstaki</i> serovar confirmed?</b>	✓	✓	✓

Logos: INSA, tbi, INRA, CFA, USJ, etc.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

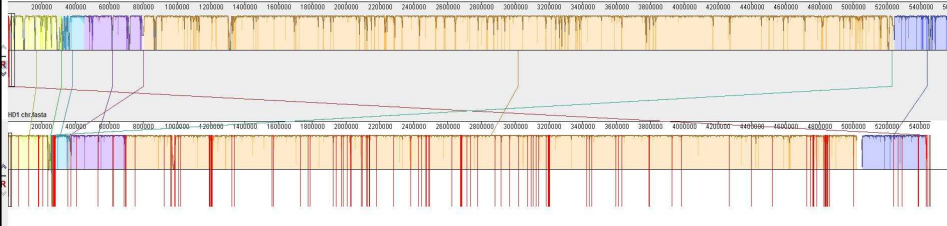
10


IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)



## Results: General WGS data

Based on previous sequencing data....






This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

11


IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)



## Results: Phylogenetic analysis

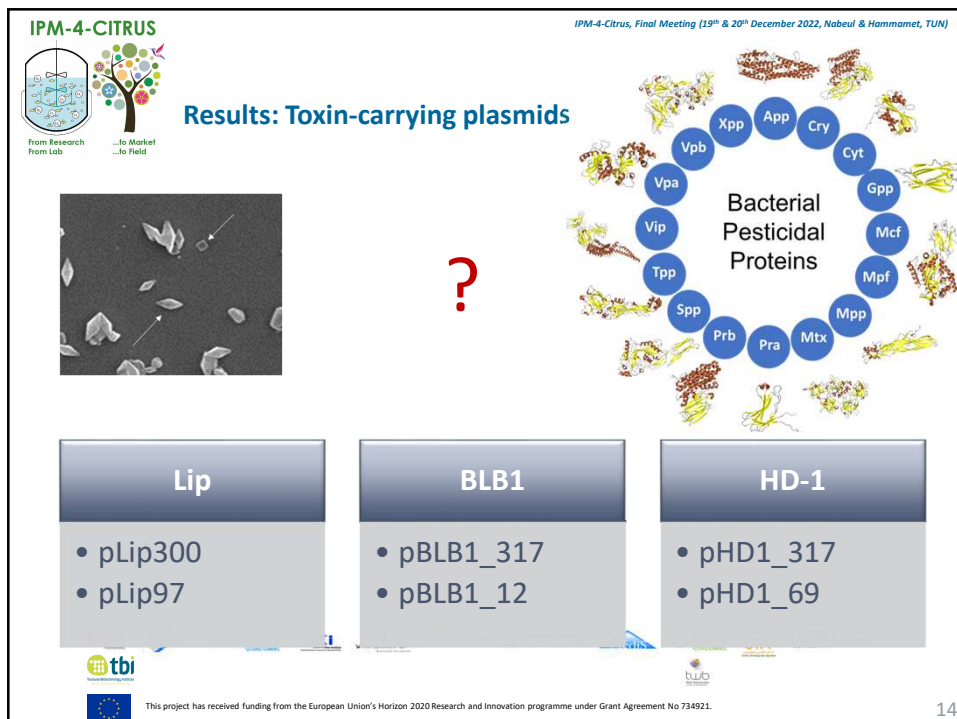
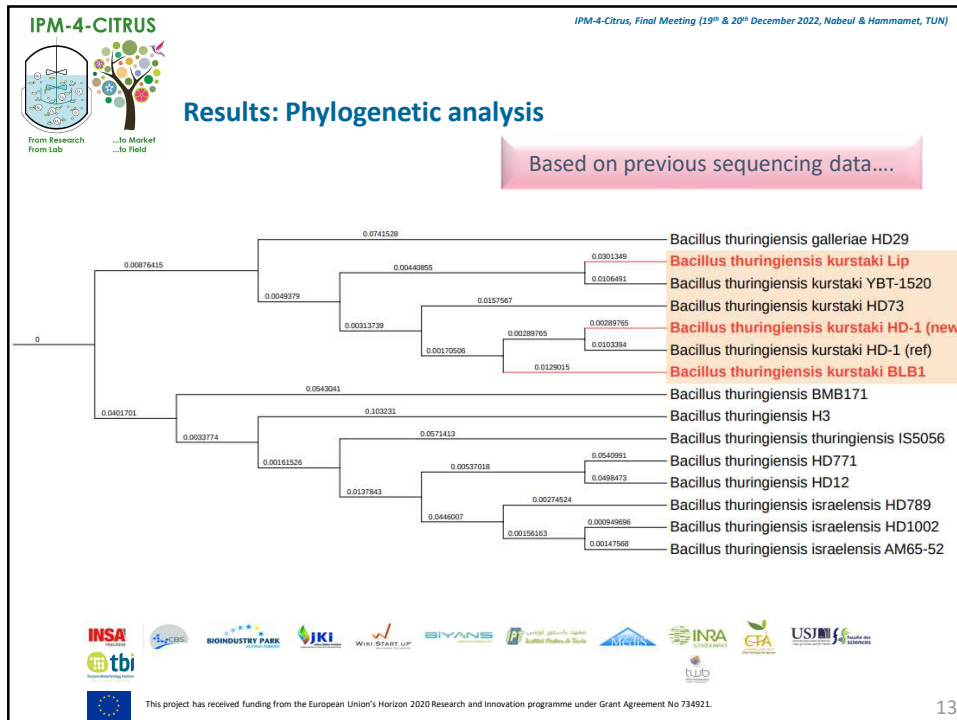
**Aim:**

See how closely the strains are related to each other and to other *Bacillus thuringiensis* strains from the database



This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

12



**IPM-4-CITRUS** IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

**Results:  $\delta$ -endotoxins genes**

Based on previous sequencing data....

Crystal shape	$\delta$ -endotoxin-coding genes	HD-1	BLB1	Lip
Bipyramidal	<i>cry1Aa</i>	1	1	1
	<i>cry1Ab</i>	1	1	1
	<i>cry1Ac</i>	1	1	2
	<i>cry1la</i>	1	1	1
Cubic	<i>cry2Aa</i>	1	1	1
	<i>cry2Ab</i>	1	1	1
<b>Total number of <math>\delta</math>-endotoxin <i>cry</i> genes</b>		<b>6</b>	<b>6</b>	<b>7</b>

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921. 15

**IPM-4-CITRUS** IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)

**Results:  $\delta$ -endotoxins genes**

Based on previous sequencing data....

pLip300  
 pHD1-317  
 pBLB1-317

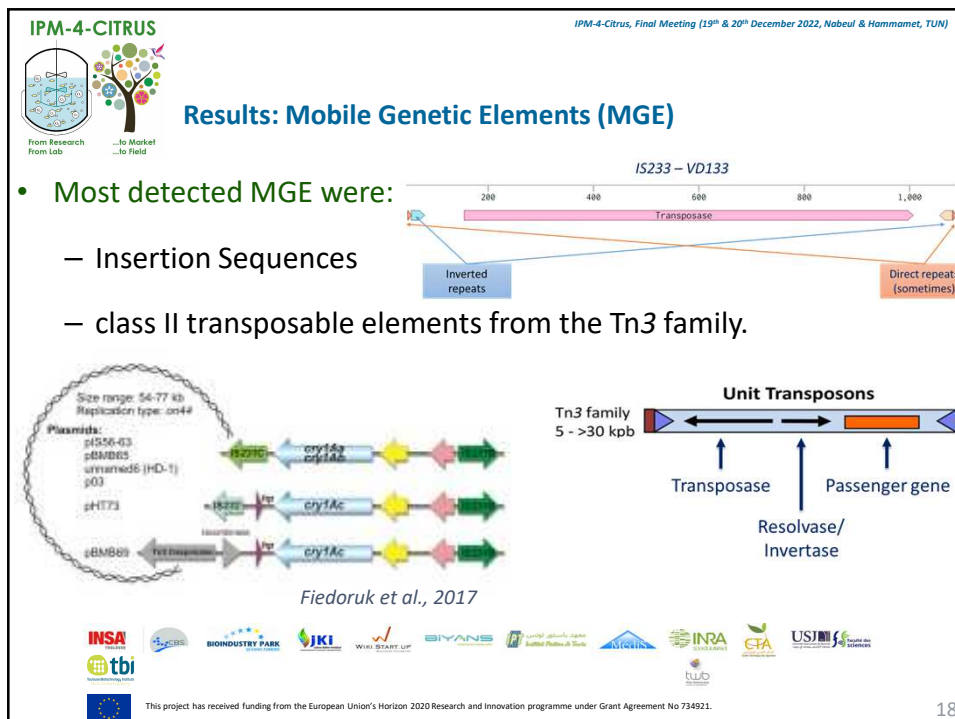
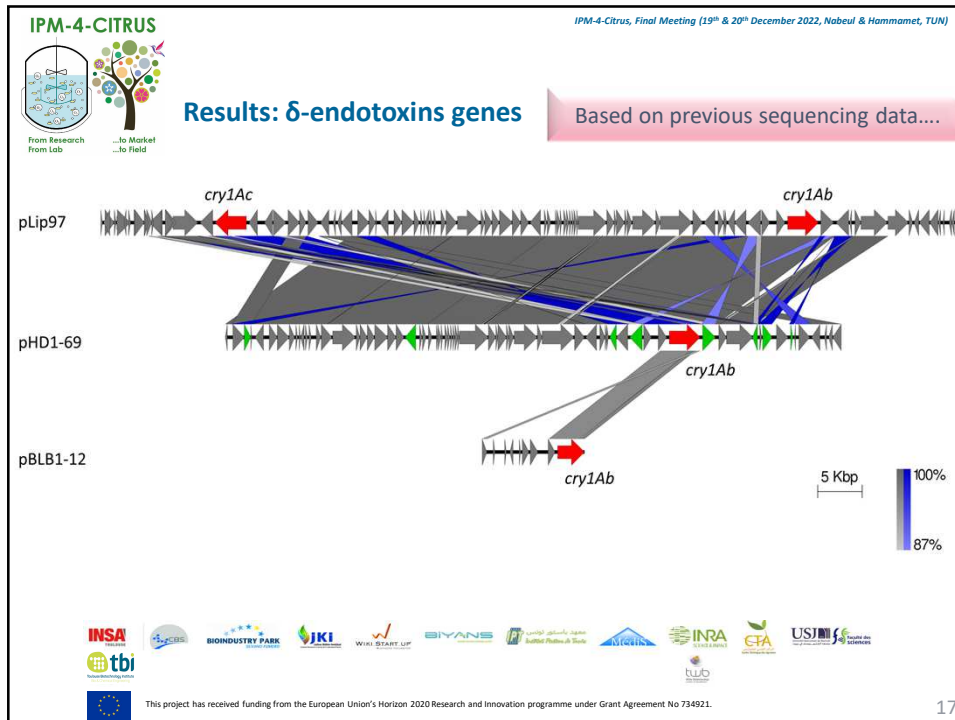
*cry1Aa*  
*cry1Ab*  
*cry1Ac*  
*cry1la*  
*cry2Aa*  
*cry2Ab*

5 Kbp  
 100%  
 73%


■  $\delta$ -endotoxin-coding genes  
■ Mobile genetic elements

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921. 16





IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)




From Research  
From Lab

...to Market  
...to Field

## Results: Mobile Genetic Elements (MGE)

Based on previous sequencing data....

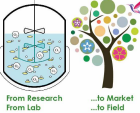
- One prophage was detected in:
  - HD1 → YES
  - Lip → YES
  - BLB1 → NO



This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

19

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)




From Research  
From Lab

...to Market  
...to Field

## Results: Predicted Antimicrobial Resistance Genes

Based on previous sequencing data....


- All 3 strains had:
  - class A Bacillus cereus Bc beta-lactamase
  - fosfomycin thiol transferase
- These resistance genes are normally found in *Bacillus* strains
- Whether they are expressed or not remains to be verified experimentally



This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

20


IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)



From Research From Lab ...to Market ...to Field

### Take home messages & Next steps

- Detailed genomic analysis of *Bacillus thuringiensis* strains:
  - A must before their mass use as biopesticides
  - Rich mobile genetic elements content
  - High potential for genetic exchange within a microbial community
- Complement bioinformatic genomic analysis by wet lab data:
  - $\delta$ -endotoxin gene expression & protein production ?
  - Resistance to antibiotics ?



This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

21

IPM-4-CITRUS IPM-4-Citrus, Final Meeting (19<sup>th</sup> & 20<sup>th</sup> December 2022, Nabeul & Hammamet, TUN)



From Research From Lab ...to Market ...to Field

# THANK YOU!



This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

22