EMBO 2025: SATELLITE WORKSHOP

BIODIVERSITY GENOMICS

# Genomic and functional study of *Bacillus* endophytes from plants with different lifestyles

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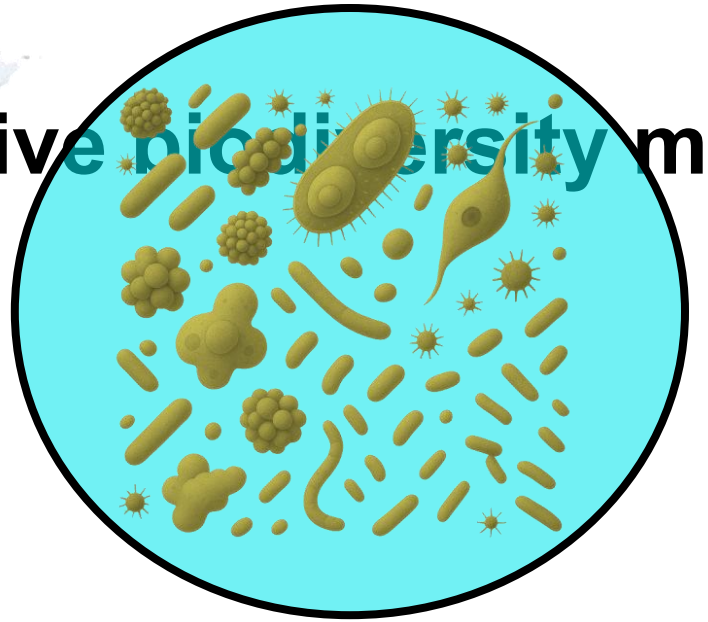
SARRIS-LAB

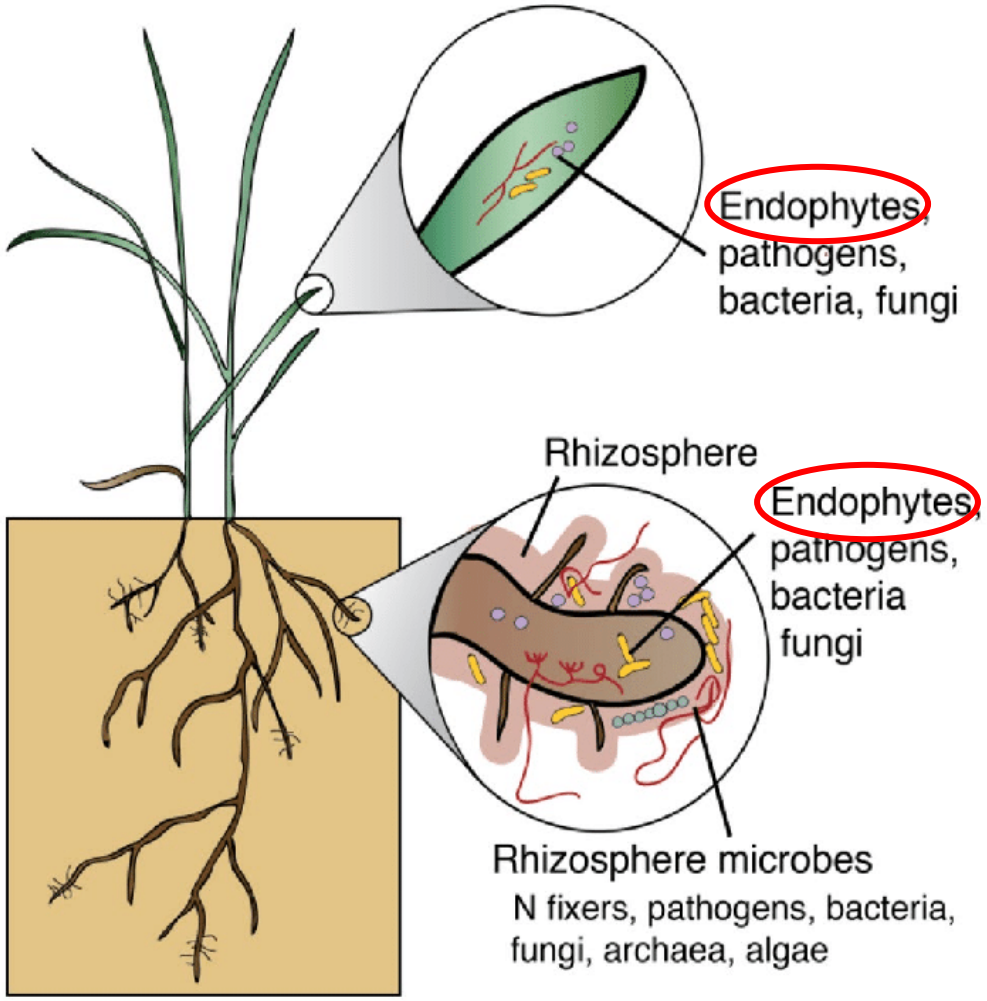
BIOCONTROL
Microbiome & Metabolites in Agriculture

’s think about someth

eive biodiversity most of the times?



# Endophytic Microbes




(Farrer, et al., 2022)

- **Non-pathogenic**
- **Plant growth-promotion**
- Improved **resistance against biotic** and **abiotic stress** conditions
- Production of **bioactive compounds**

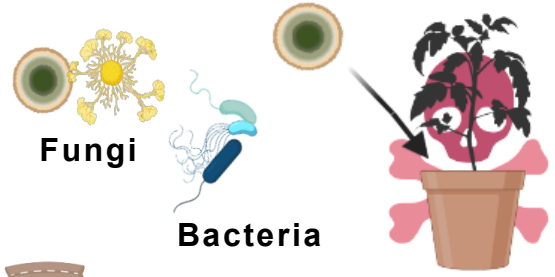
(Bulgarelli, D. et al., 2013)

(Gouda, S. et al., 2016) (Santoyo, G. et al., 2016)

# Endophytes as a solution for a sustainable agriculture

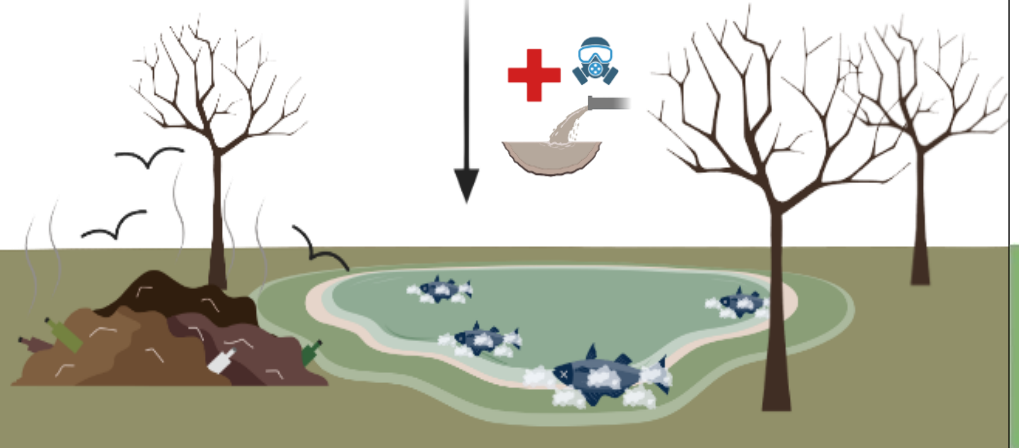
## 1) The challenge of modern agriculture

➢ **Fight against Pathogens**

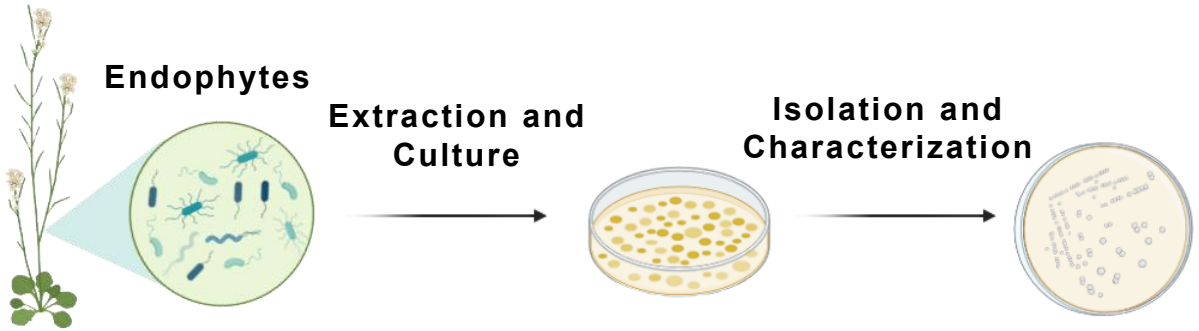




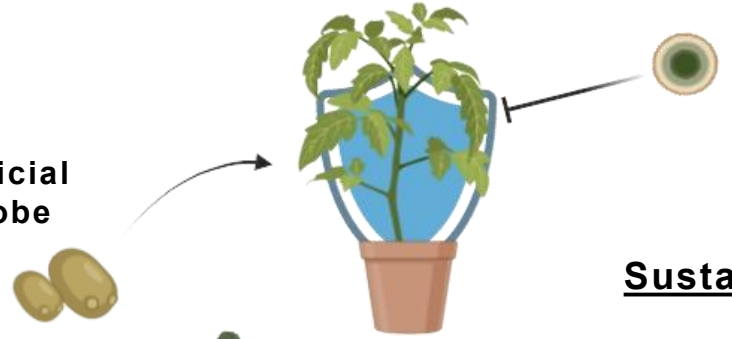
**Pollution**



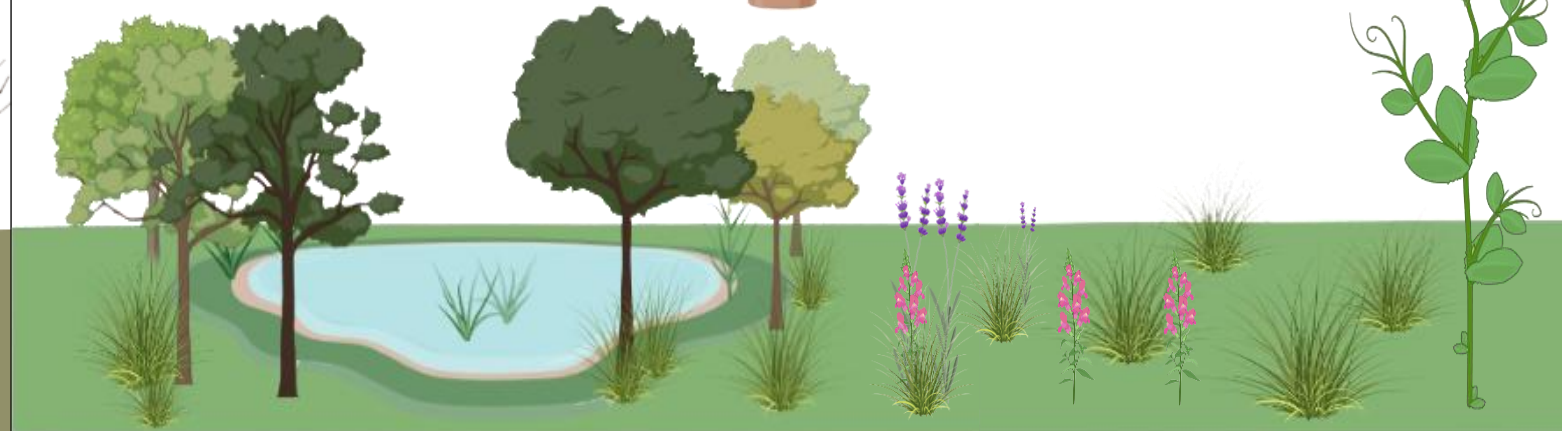
## 2) Exploring the microbial arsenal of plants




➢ **Identification of BENEFICIAL MICROBES which protect plants from pathogens**




**Sustainability**



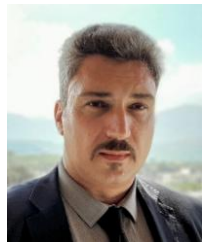
# Scientific Questions

- Do the **endophytic microbes** of plants that **live in extreme environments** have a role in **stress tolerance**?

- Could the **endophytic microbes of crop wild relatives** (**CWRs)** be a good **source of beneficial microbes** for crops contribute to **abiotic** and **biotic stress tolerance**?

- **Approach: Phylogenetics, Comparative Genomics** and **Functional Analysis** of the endophytes to get unique insights in uncovering **genomic diversity** and identify **novel traits crucial for future applications**

# Sampling of halophytes and olive trees in Crete and Chrisi island



**Prof. P. Sarris**
Group Leader

## ▸ Plant species collected from Crete

*Cakile maritima*

*Matthiola tricuspidata*

*Crithmum maritimum*

*Olea europaea*

**Mr N. Arapitsas**
PhD student

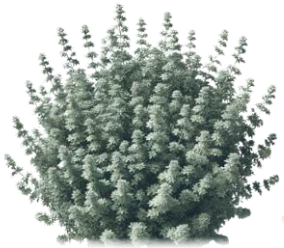
**Dr Ch. Christakis**
former Post-Doc

**Dr S. Paragkamian**
Post-Doc

**Mr M. Avramakis**
Botanist

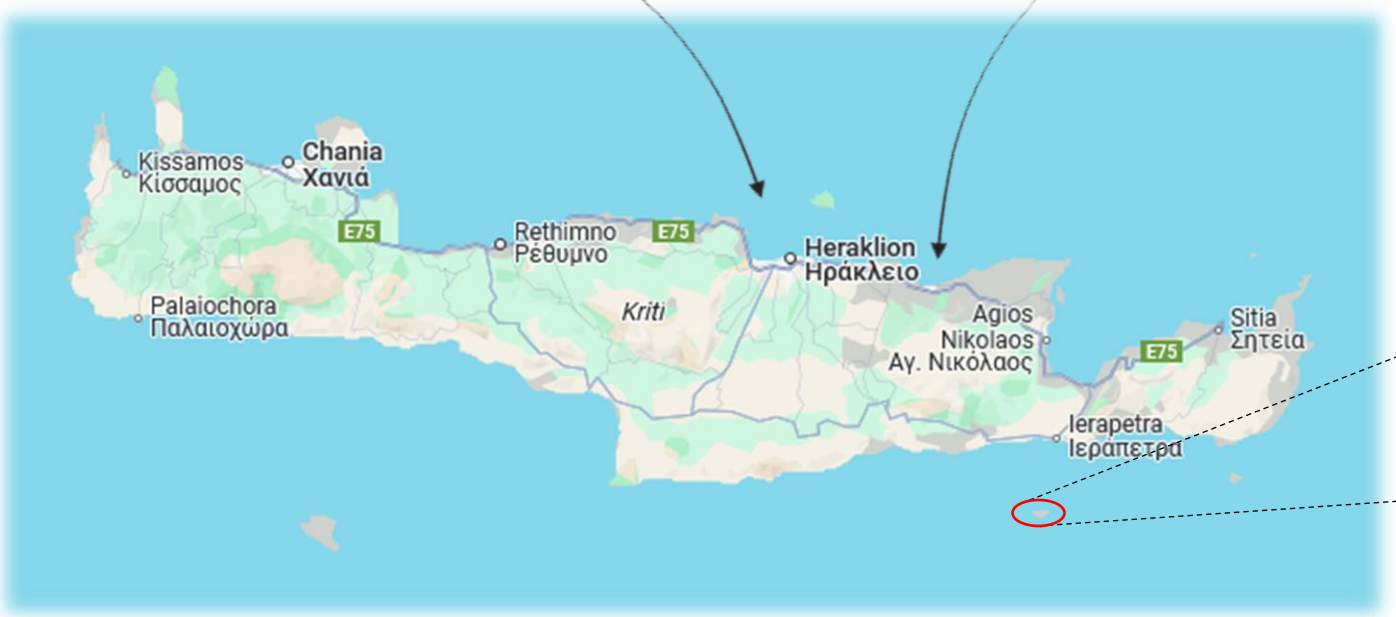
## ▸ Plant species collected from Chrisi island

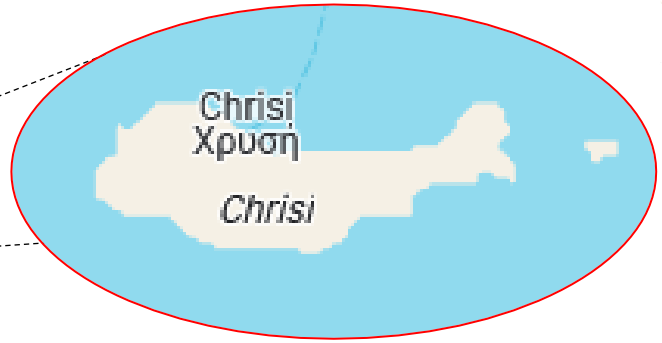
*Thymus capitatus*



*Atriplex halimus*

*Tetraena alba*




*Frankenia sp.*

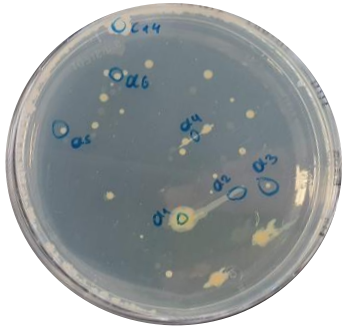
*(Christakis, et al., 2021)*

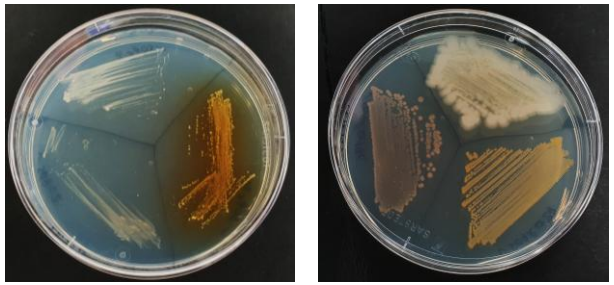
*(source: Google Maps)*

# Isolation, identification and characterization of endophytes

**Media**

- **NA**
- **R2A**
- **NA1/2**





**Isolation and characterization of endophytic isolates**

Root/Leaf extract

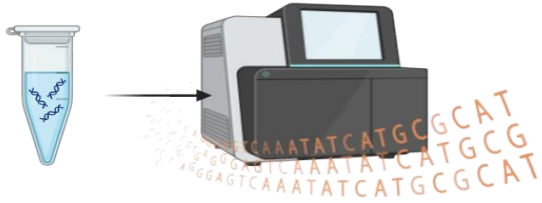
Grinding

Surface sterilization

Leaves

Roots

**Bio-bank of Isolates**



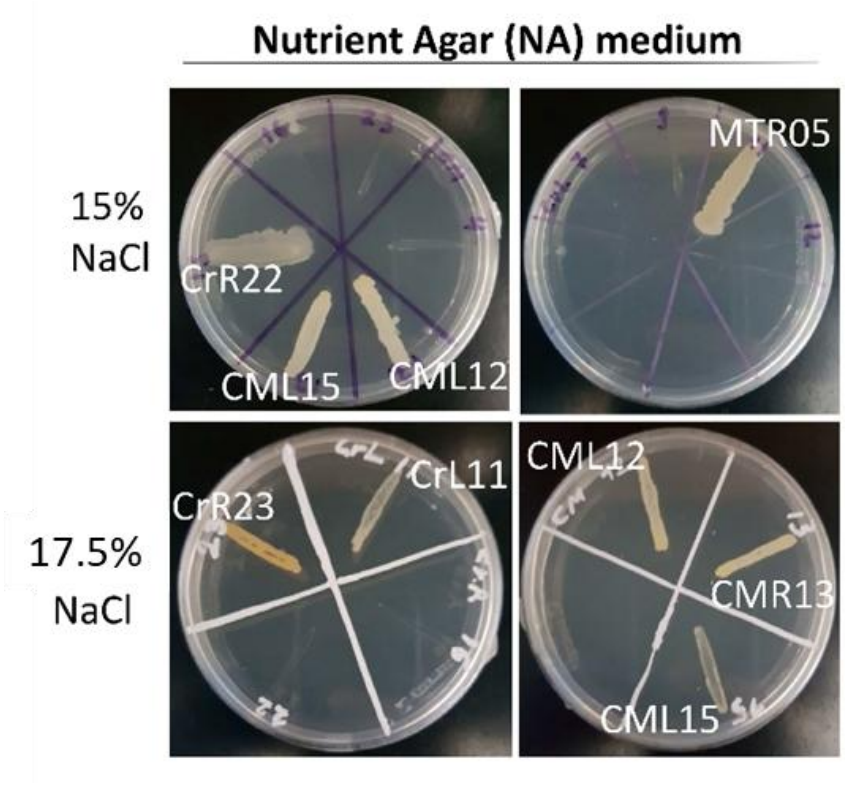

**Identification based on 16S rDNA**

*(part of the scheme created with BioRender.com)*

**≈ 500 isolates from halophytes and olive trees from Crete and halophytes from Chrisi island**

# Salt tolerance assays

***In vitro***




***In planta in 250mM NaCl***

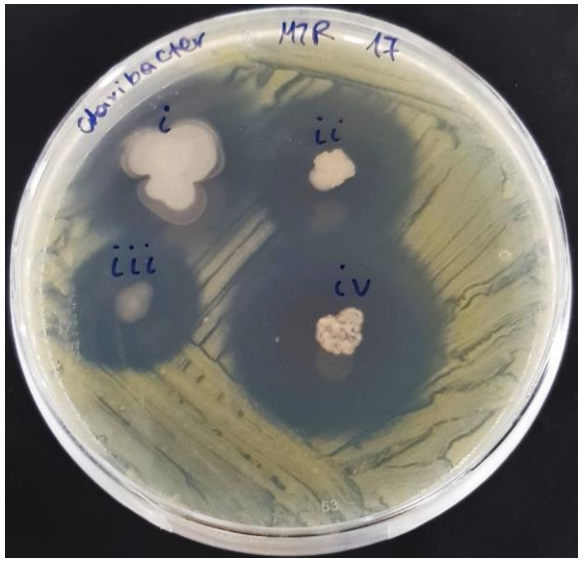
***Plants + Bacterial isolate***

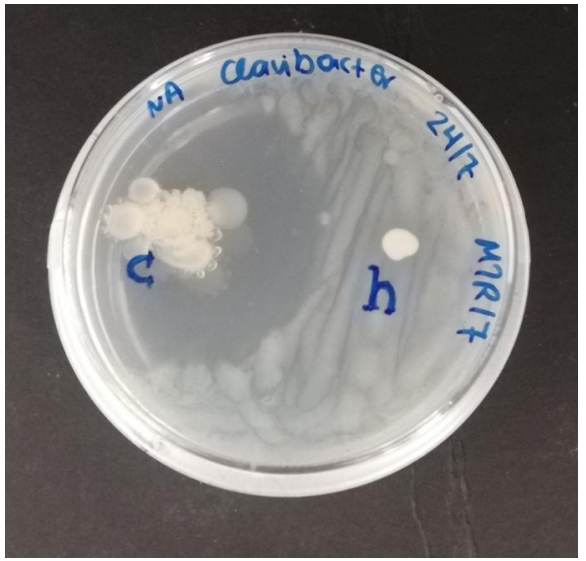
***Control plants***

***Control plants + E. coli***

# Bioassays against bacterial phytopathogens

- *Clavibacter michiganensis*
- *Paracidovorax citrulli* (former *Acidovorax citrulli*)
- *Ralstonia solanacearum*

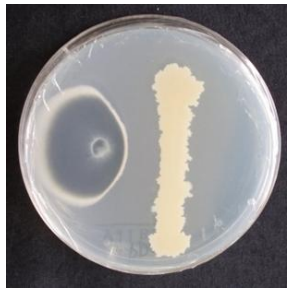


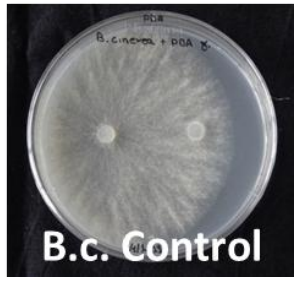
# Bioassays against phytopathogenic fungi

***Verticillium***






***Botrytis***

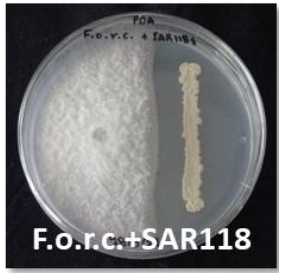


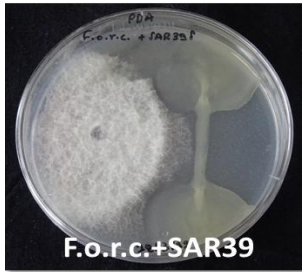




***Fusarium***



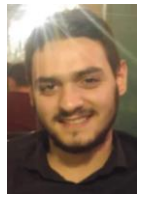



***Phytophthora***





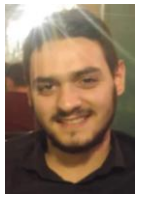



**Mr S. Soultatos**
PhD student

**Prof. Em. Markakis**
Professor


***+ Alternaria***

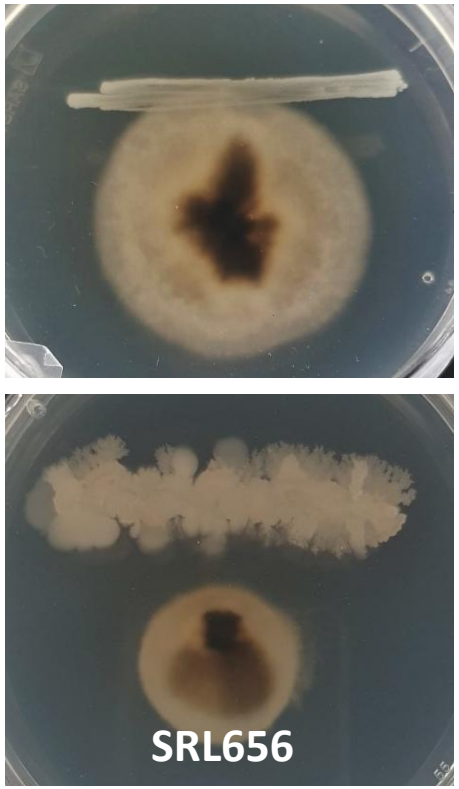
# Bioassays against phytopathogenic fungi



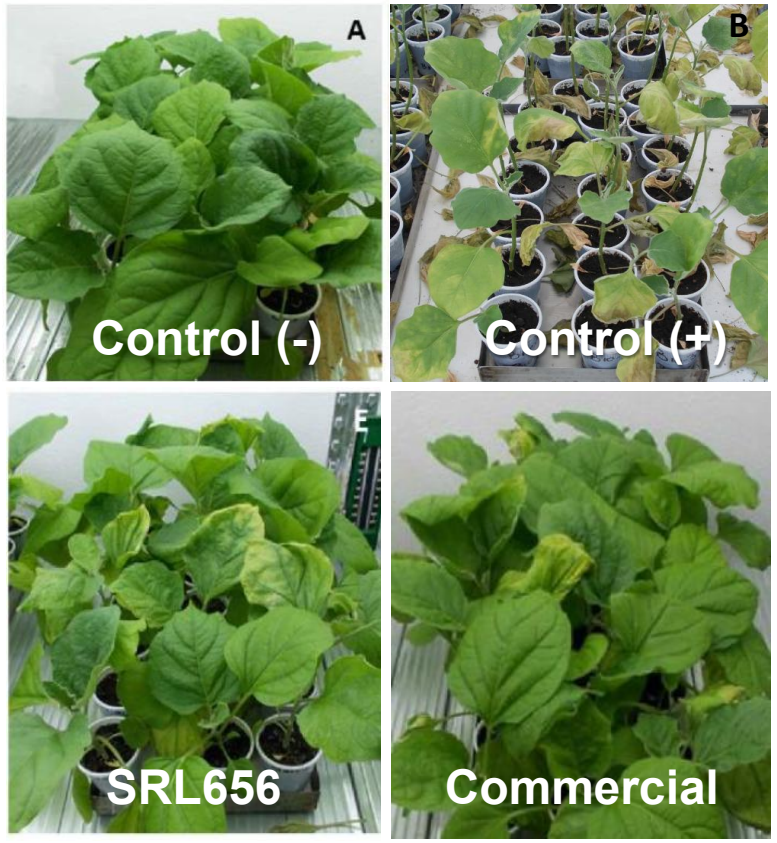
**Mr S. Soultatos**
PhD student

**Prof. Em. Markakis**
Professor

***In vitro***




***In planta***




**Symptoms in artificially infected plants with *Verticillium dahliae***

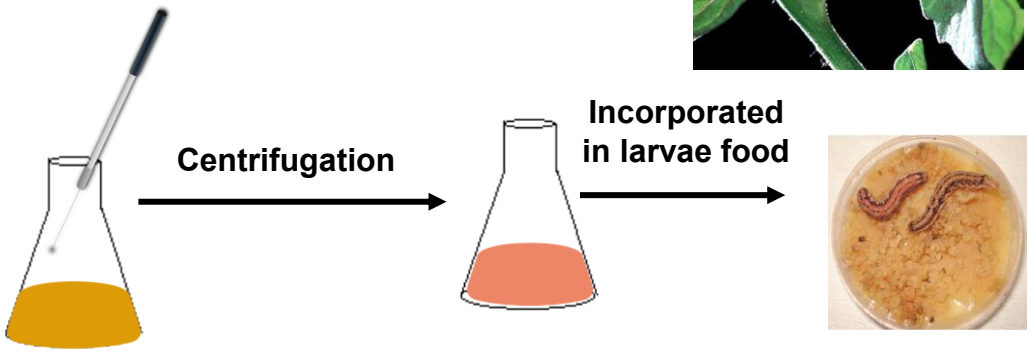
# Bioassays against insect larvae

FORTH
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

**Dr A. Kampouraki**
Postdoc

**Mr S. Mastis**
PhD student

## vs *Helicoverpa* larvae




**Control food**

**Food + bacterial isolate**

## vs *Culex pipiens* mosquito (Diptera: Culicidae)

**Control** *(0% mortality in 24hrs)*

***Bacillus* sp.** extract *(100% mortality in 24hrs)*

**Collaboration: Insects Biology – Prof. Vontas Lab**
IMBB-FORTH

# Selection of isolates for hybrid whole genome sequencing

| Isolate ID | *Species based on 16S rDNA* | Max Salt Tolerance | Inhibition against bacterial pathogens | INH zone against Verticillium dahliae (%) | INH zone against Alternaria sp. (%) | INH zone against Botrytis cinerea (%) | INH zone against Fusarium sp. (%) | Other reasons |
|---|---|---|---|---|---|---|---|---|
| SRL152 | *Bacillus thuringiensis* | 5% NaCl | | | | | | interesting phylogenetically |
| SRL163 | *Bacillus amyloliquefaciens* | 7.5% NaCl | | | | | | interesting phylogenetically |
| SRL179 | *Bacillus drentensis* | 17.5% NaCl | | | | | | |
| SRL215 | *Bacillus thuringiensis* | 5% NaCl | | | | | | interesting phylogenetically |
| SRL218 | *Bacillus thuringiensis* | 5% NaCl | | | | | | interesting phylogenetically |
| SRL221 | *Bacillus methylotrophicus synonymous* | 10% NaCl | | | | | | interesting phylogenetically |
| SRL224 | *Bacillus thuringiensis* | 5% NaCl | | | | | | interesting phylogenetically |
| SRL244 | *Bacillus siamensis* | 7.5% NaCl | | | | | | interesting phylogenetically |
| SRL266 | *Peribacillus frigoritolerans strain WS2-1* | 17.5% NaCl | | | | 18.92 | 24.68 | |
| SRL335 | *Cytobacillus oceanisediminis 2691* | | | 45.45 | 25.00 | | | |
| SRL337 | *Bacillus salacetis strain VS-19* | 17.5% NaCl | | | | | | |
| SRL340 | *Peribacillus simplex strain BS20* | | | | 24.24 | | | |
| SRL342 | *Paenibacillus xylanexedens strain 3-4T* | | | | 33.33 | | | |
| SRL368 | *Bacillus cereus strain Xuyi_401_1* | 17.5% NaCl | | 37.33 | | 35.14 | 28.57 | effective against *Culex pipiens* and *Helicoverpa* |
| SRL369 | *Bacillus subtilis WN-1* | 10% NaCl | | | | 29.73 | | |
| SRL374 | *Bacillus siamensis strain LB146* | 10% NaCl | | 76.00 | 33.33 | 45.95 | 61.04 | |
| SRL379 | *Bacillus amyloliquefaciens strain PD2* | 7,5% NaCl | Inhibition against Clavibacter | 68.00 | 30.30 | 43.24 | 53.25 | |
| SRL389 | *Bacillus simplex strain ILQ109* | 7,5% NaCl | | 40.00 | | 19.74 | | |
| SRL398 | *Paenibacillus xylanexedens strain 3-4T* | 5% NaCl | Inhibition against Clavibacter | | | | 22.86 | |
| SRL543 | *Bacillus infantis* | 10% NaCl | | Large | | | | |
| SRL544 | *Bacillus haikouensis* | 15% NaCl | | | | | | only one other genome and only as a Scaffold |
| SRL571 | *Bacillus altitudinis* | | Inhibition against Clavibacter | Large | | | | |
| SRL656 | *Bacillus licheniformis* | 5% NaCl | | Large | | | | |
| SRL658 | *Bacillus sonorensis* | | | Large | | | | |
| SRL662 | *Bacillus licheniformis* | | | Large | | | | |

# Selection of isolates for hybrid whole genome sequencing

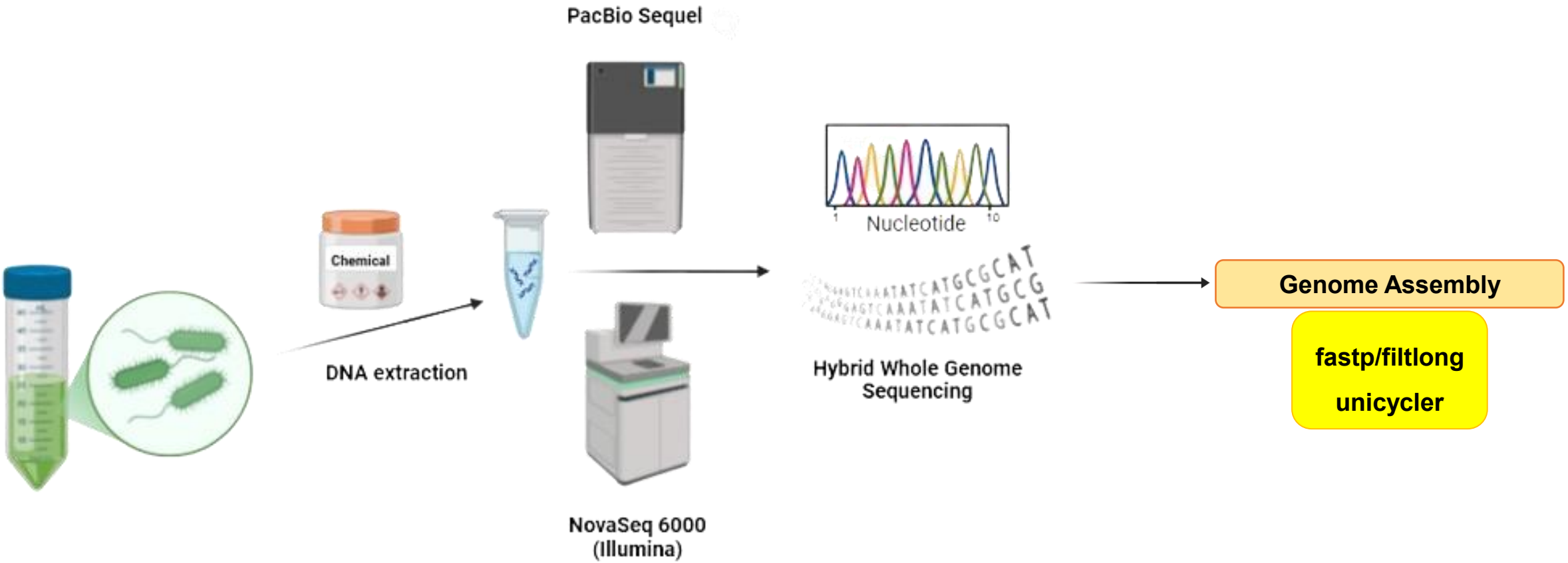
**<u>Selection criteria</u>**:

- **Salt tolerance**
- **Inhibition of plant pests and pathogens**
- **Limited online genome availability**

- **25 endophytic isolates** from olive trees and halophytes from Crete and Chrisi island

## Focus on the class Bacilli

# Hybrid whole genome sequencing combining short and long reads

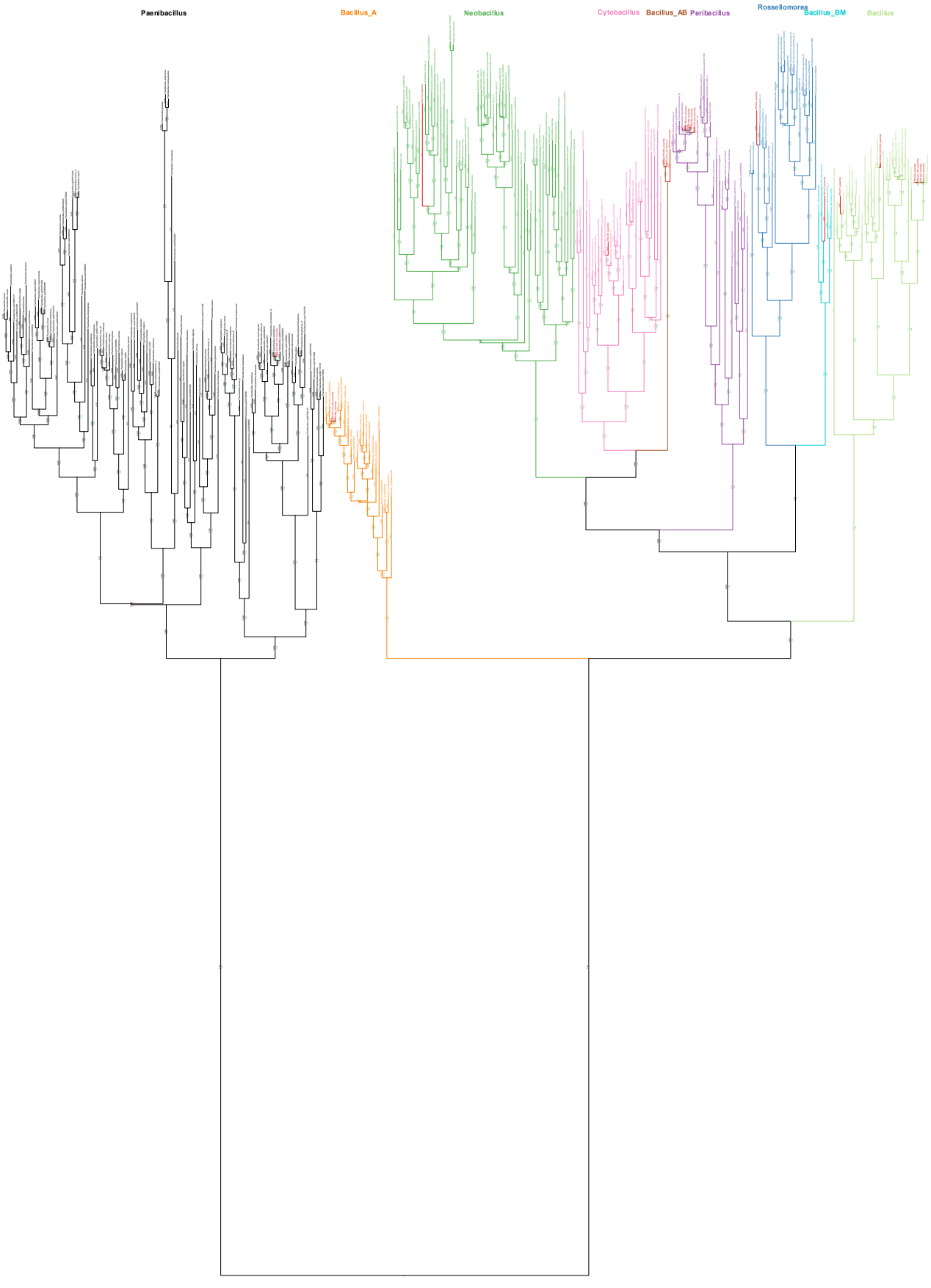


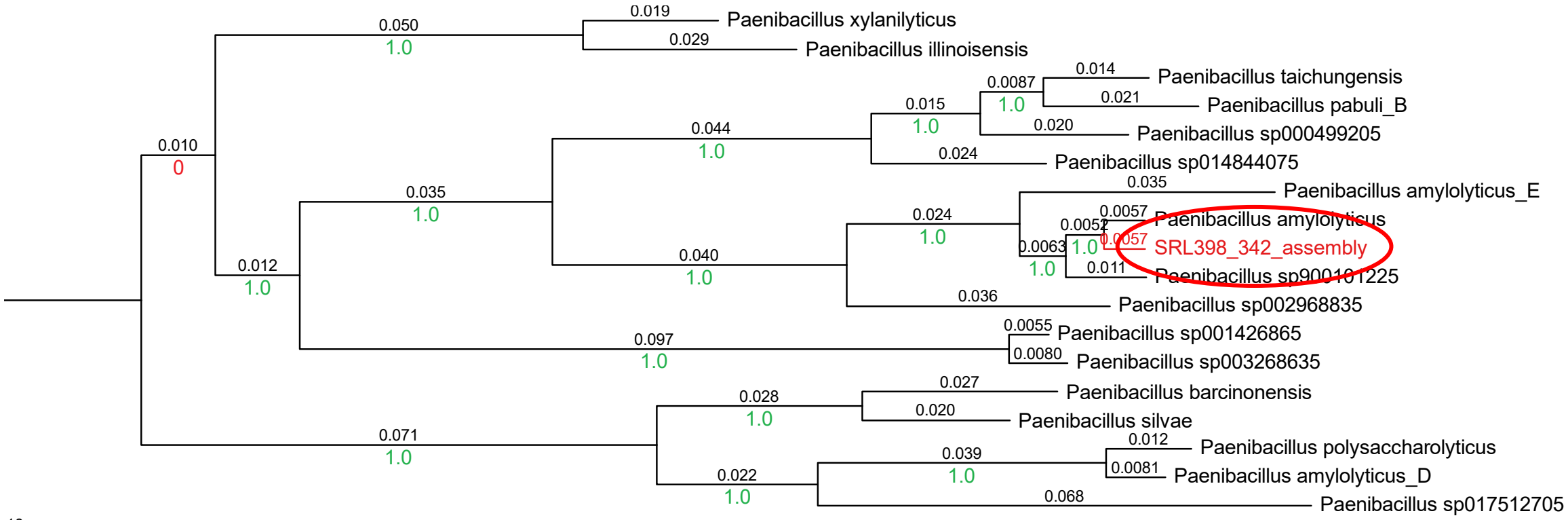

*(created with BioRender.com)*

# Identification of the isolates using FastANI

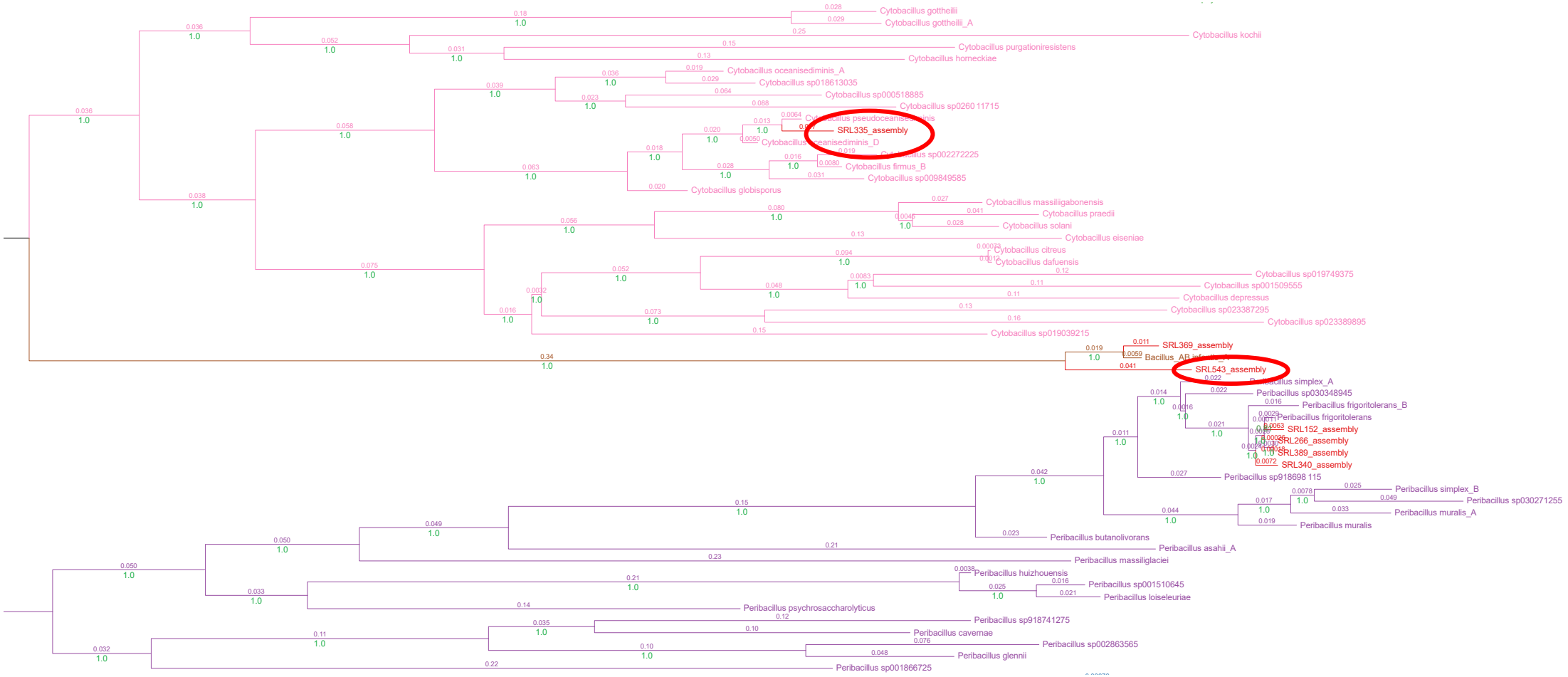
| Isolate ID | Origin | Species wGTDBk (WGS) | GTDBk FastANI % |
|---|---|---|---|
| SRL152 | Olive tree | *Peribacillus frigoritolerans* | 97.47 |
| SRL163 | Olive tree | *B. velezensis* | 99.04 |
| SRL179 | Olive tree | *Neobacillus jeddahensis* | **80.14** |
| SRL215 | Olive tree | *Bacillus_A thuringiensis_S* | 99.21 |
| SRL218 | Olive tree | *B. thuringiensis* | 99.07 |
| SRL221 | Olive tree | *B. velezensis* | 99.04 |
| SRL224 | Olive tree | *Bacillus thuringiensis* | 99.22 |
| SRL244 | Olive tree | *B. velezensis* | 99.03 |
| SRL266 | Halophyte from Chrysi island | *Peribacillus frigoritolerans* | 96.51 |
| SRL335 | Halophyte from Chrysi island | *Cytobacillus oceanisediminis* | 95.33 |
| SRL337 | Halophyte from Chrysi island | *Bacillus salacetis* | **83.69** |
| SRL340 | Halophyte from Chrysi island | *Peribacillus frigoritolerans* | 95.51 |
| SRL342 | Halophyte from Chrysi island | *Paenibacillus sp001955855* | 96.08 |
| SRL368 | Halophyte from Chrysi island | *Bacillus thuringiensis* | 99.32 |
| SRL369 | Halophyte from Chrysi island | *Bacillus infantis* | 98.95 |
| SRL374 | Halophyte from Chrysi island | *Bacillus velezensis* | 99.04 |
| SRL379 | Halophyte from Chrysi island | *Bacillus velezensis* | 99.03 |
| SRL389 | Halophyte from Chrysi island | *Peribacillus frigoritolerans* | 96.49 |
| SRL398 | Halophyte from Chrysi island | *Paenibacillus sp001955855* | 96.08 |
| SRL543 | Halophyte from Crete | *Bacillus infantis* | **90.14** |
| SRL544 | Halophyte from Crete | *Rossellomorea (previously Bacillus) haikouensis* | 99.99 |
| SRL571 | Halophyte from Crete | *Bacillus altitudinis* | 97.98 |
| SRL656 | Halophyte from Crete | *Bacillus paralicheniformis* | 98.85 |
| SRL658 | Halophyte from Crete | *B. paralicheniformis* | 98.85 |
| SRL662 | Halophyte from Crete | *Bacillus paralicheniformis* | 98.85 |

## Four putative novel species


Paenibacillus
Bacillus_A
Neobacillus
Cytobacillus
Bacillus_AB
Peribacillus
Rossellomorea
Bacillus_BM
Bacillus


Paenibacillus xylanilyticus
Paenibacillus illinoisensis
Paenibacillus taichungensis
Paenibacillus pabuli_B
Paenibacillus sp000499205
Paenibacillus sp014844075
Paenibacillus amylolyticus_E
Paenibacillus amylolyticus
SRL398_342_assembly
Paenibacillus sp900101225
Paenibacillus sp002968835
Paenibacillus sp001426865
Paenibacillus sp003268635
Paenibacillus barcinonensis
Paenibacillus silvae
Paenibacillus polysaccharolyticus
Paenibacillus amylolyticus_D
Paenibacillus sp017512705
Paenibacillus

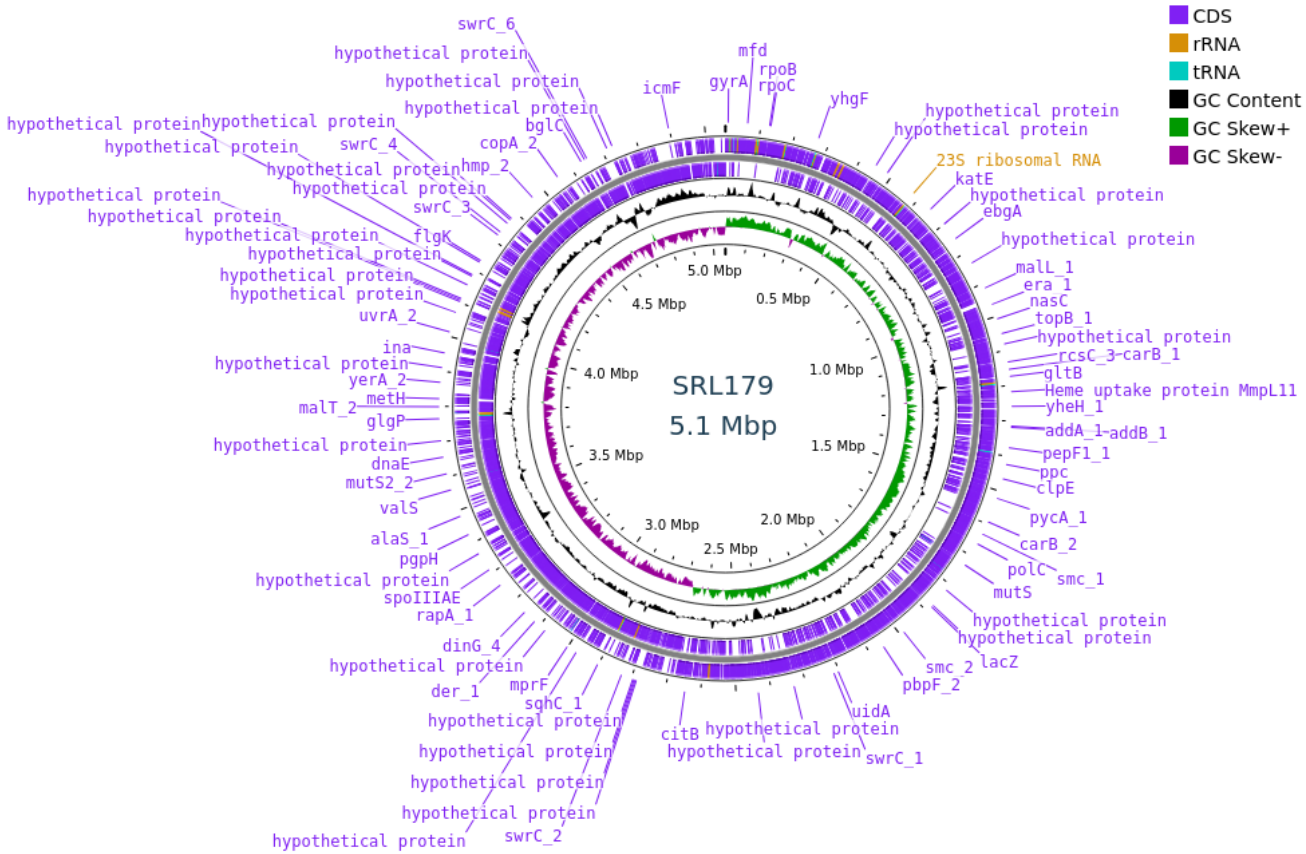

Neobacillus sp030403775
Neobacillus sp023512145
Neobacillus soli
Neobacillus sp002567005
Neobacillus sp000686805
Neobacillus sp007678255
Neobacillus sp019219025
Neobacillus sp018613105
SRL179_assembly
Neobacillus cucumis_A
Neobacillus sp009928415
Neobacillus sp002585305
Neobacillus drentensis_A
Neobacillus cucumis
Neobacillus jeddahensis
Neobacillus sp005154805
Neobacillus sp030123405
Neobacillus sp0 11250555
Neobacillus bataviensis
Neobacillus rhizosphaerae
Neobacillus novalis
Neobacillus vireti
Neobacillus sp030818775
Neobacillus sp030915525
Neobacillus pocheonensis
Neobacillus ginsengisoli
Neobacillus niacini_B
Neobacillus niacini_F
Neobacillus sp03 1177865
Neobacillus sp030123065
Neobacillus niacini
Neobacillus niacini_A
Neobacillus niacini_D
Neobacillus sp002272245
Neobacillus sp013396335
Neobacillus niacini_C
Neobacillus sp030348765
Neobacillus sp900103525
Neobacillus sp029256785
Neobacillus sp001940785
Neobacillus sp946151075
Neobacillus massiliamazoniensis
Neobacillus sp001866655
Neobacillus salipaludis
Neobacillus rhizophilus
Neobacillus citreus
Neobacillus niacini_E
Neobacillus thermocopriae
Neobacillus dielmonensis
Neobacillus muris
Neobacillus mesonae
Neobacillus kokaensis
Neobacillus mesonae_A
Neobacillus renqingensis
Neobacillus rubiinfantis
Neobacillus paridis
Neobacillus fumarioli
Neobacillus endophyticus
Neobacillus

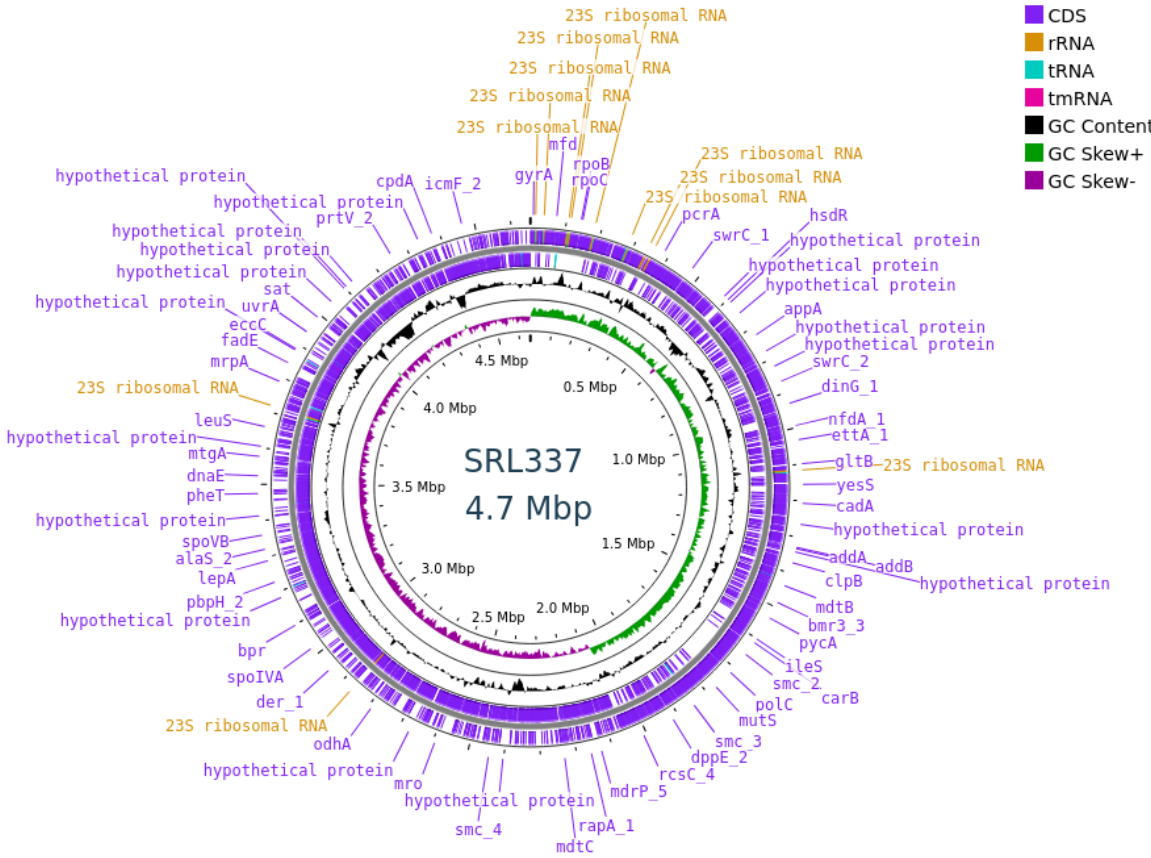



Cytobacillus Bacillus_AB Peribacillus


Rossellomorea marisflavi_A
Rossellomorea marisflavi_B
SRL544_assembly
Rossellomorea haikouensis_A
Rossellomorea aquimaris_A
Rossellomorea enclensis
Rossellomorea sp903645295
Rossellomorea aquimaris_F
Rossellomorea aquimaris
Rossellomorea aquimaris_E
Rossellomorea sp019334305
Rossellomorea sp017303375
Rossellomorea sp002561455
Rossellomorea vietnamensis_B
Rossellomorea vietnamensis_A
Rossellomorea arthrocnemi
Rossellomorea sp005938125
Rossellomorea aquimaris_B
Rossellomorea sp947488365
Rossellomorea sp008364765
Rossellomorea sp0129 11895
Rossellomorea sp0 11398925
Bacillus_BM sp000181495
Bacillus_BM sp
SRL337_assembly
Bacillus_BM vietnamensis_A
Bacillus_BM sp003316765
Bacillus gobiensis
Bacillus xiamenensis
SRL571_assembly
Bacillus altitudinis
Bacillus stratosphericus_B
Bacillus pumilus_N
Bacillus pumilus
Bacillus safensis
Bacillus pumilus_P
Bacillus pumilus_G
Bacillus glycinifermentans
Bacillus sonorensis
Bacillus sonorensis_A
Bacillus swezeyi
Bacillus paralicheniformis
SRL662_656_658_assembly
Bacillus sp013155385
Bacillus halotolerans
Bacillus mojavensis
Bacillus subtilis_G
Bacillus vallismortis
Bacillus inaquosorum
Bacillus stercoris
Bacillus sp010093085
Bacillus atrophaeus
Bacillus sp018613535
SRL379_assembly
SRL221_244_assembly
SRL163_assembly
SRL374_assembly
Bacillus velezensis
Rossellomorea
Bacillus_BM
Bacillus

# Genome maps of the 4 putative new species



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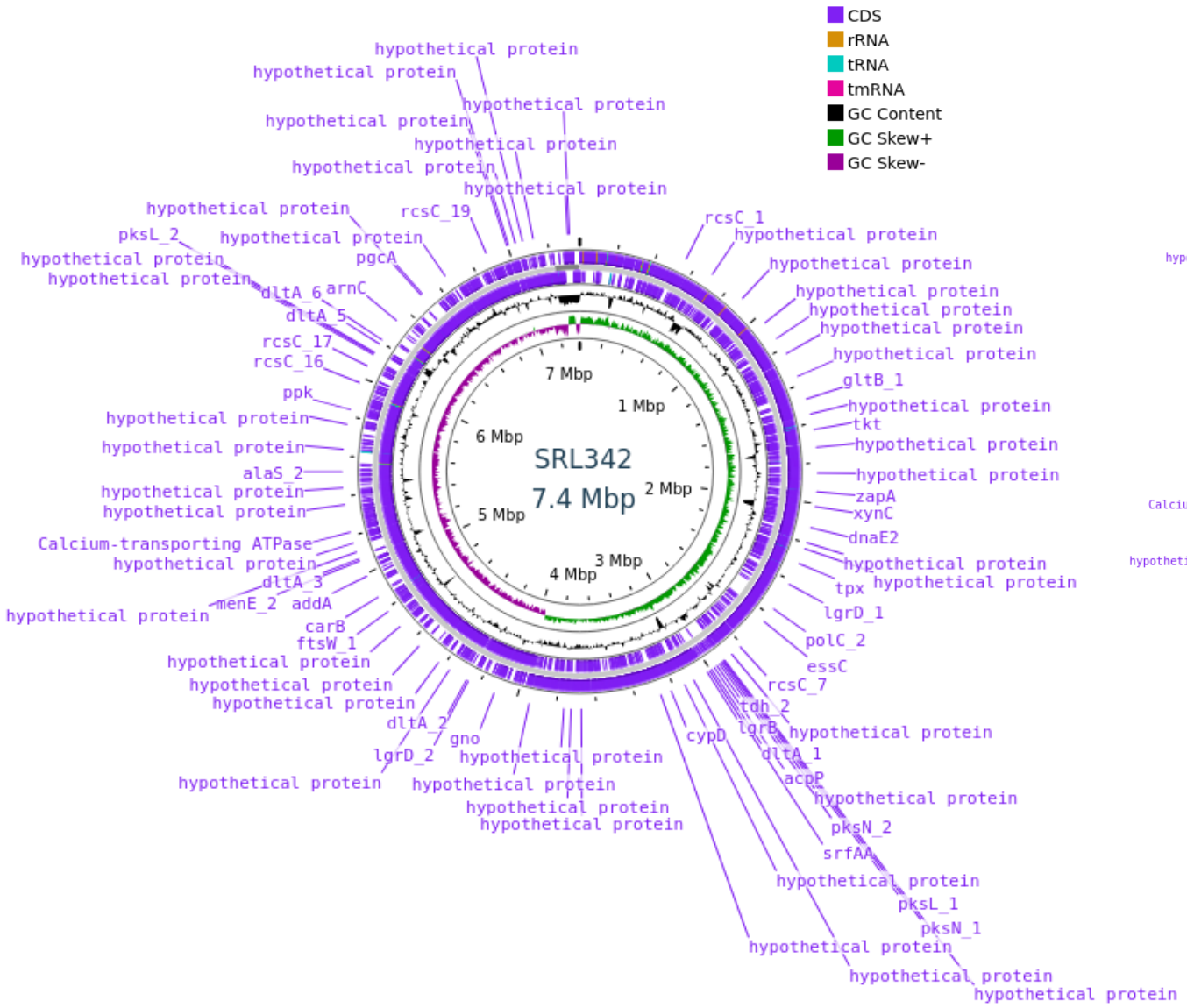


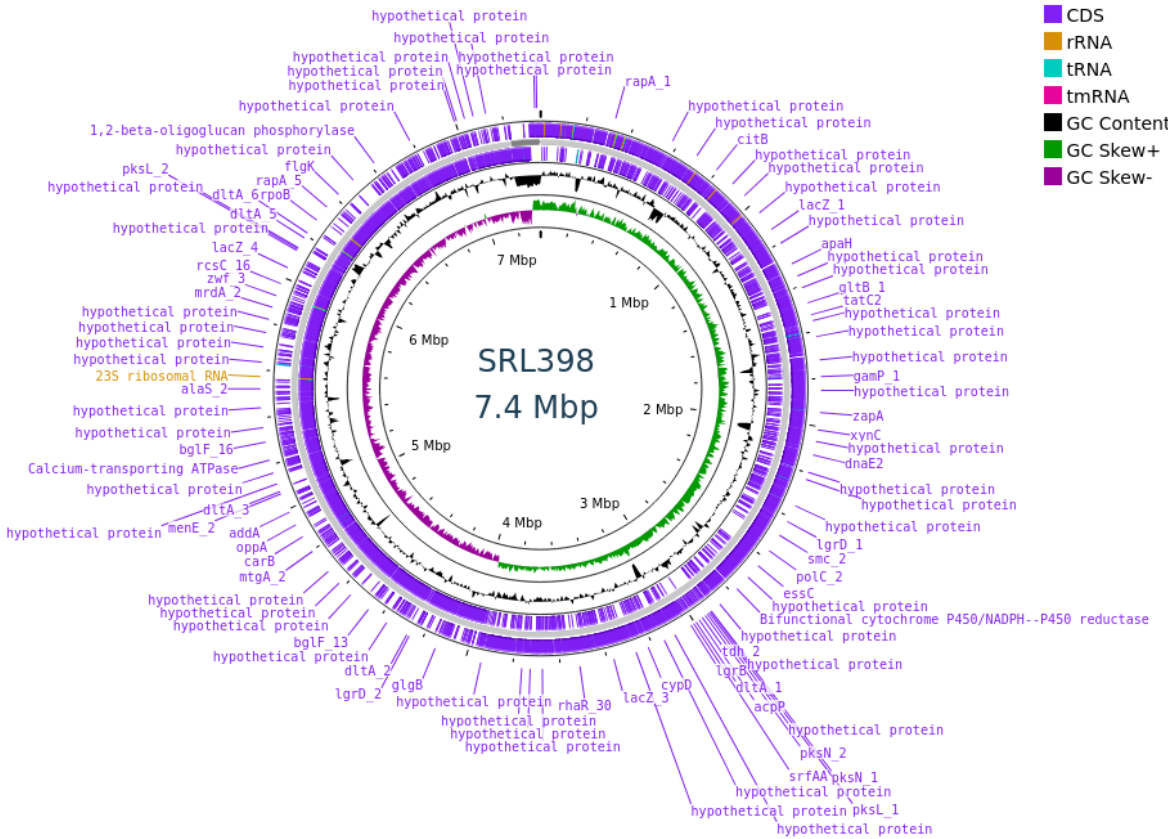

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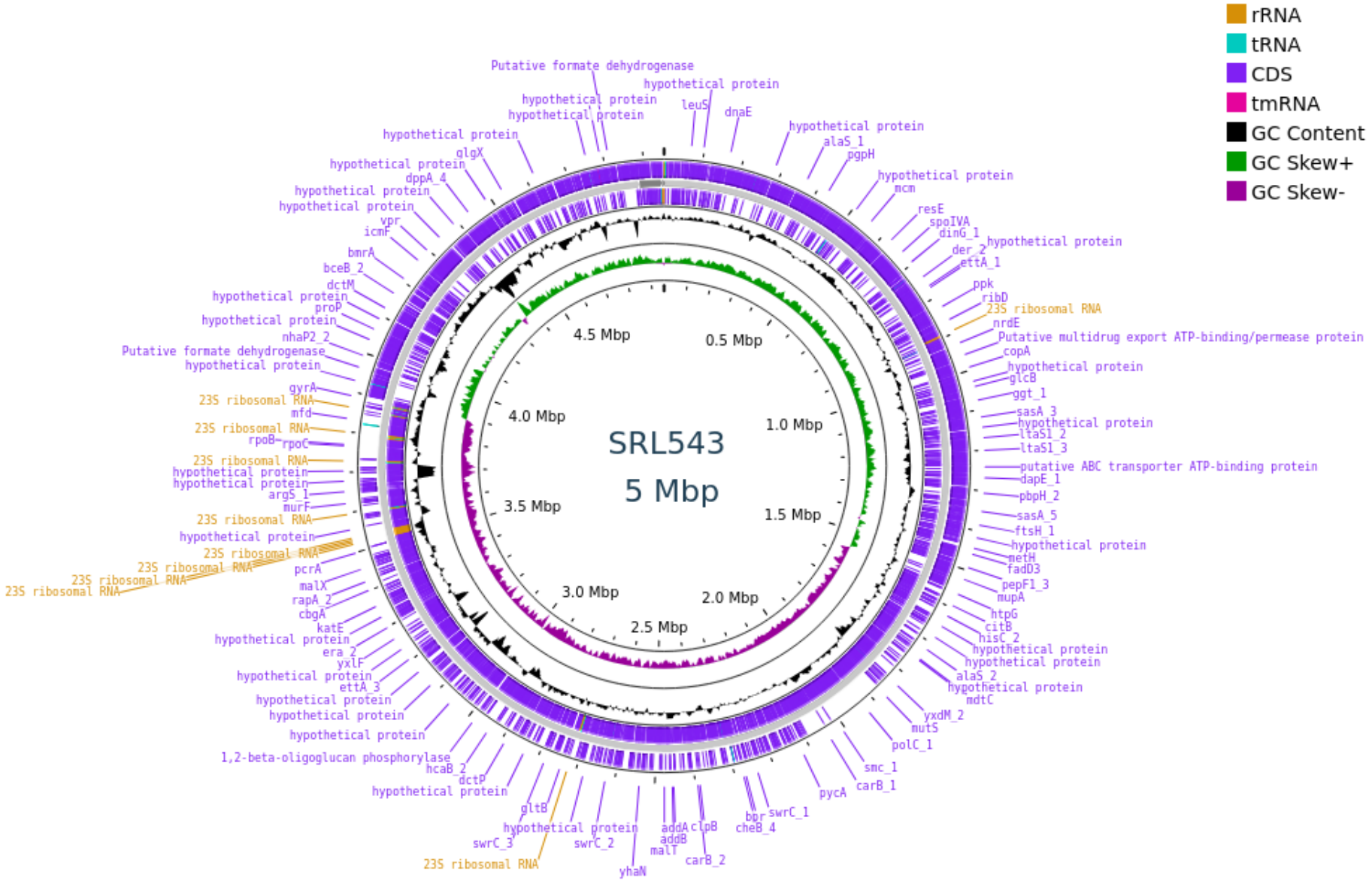
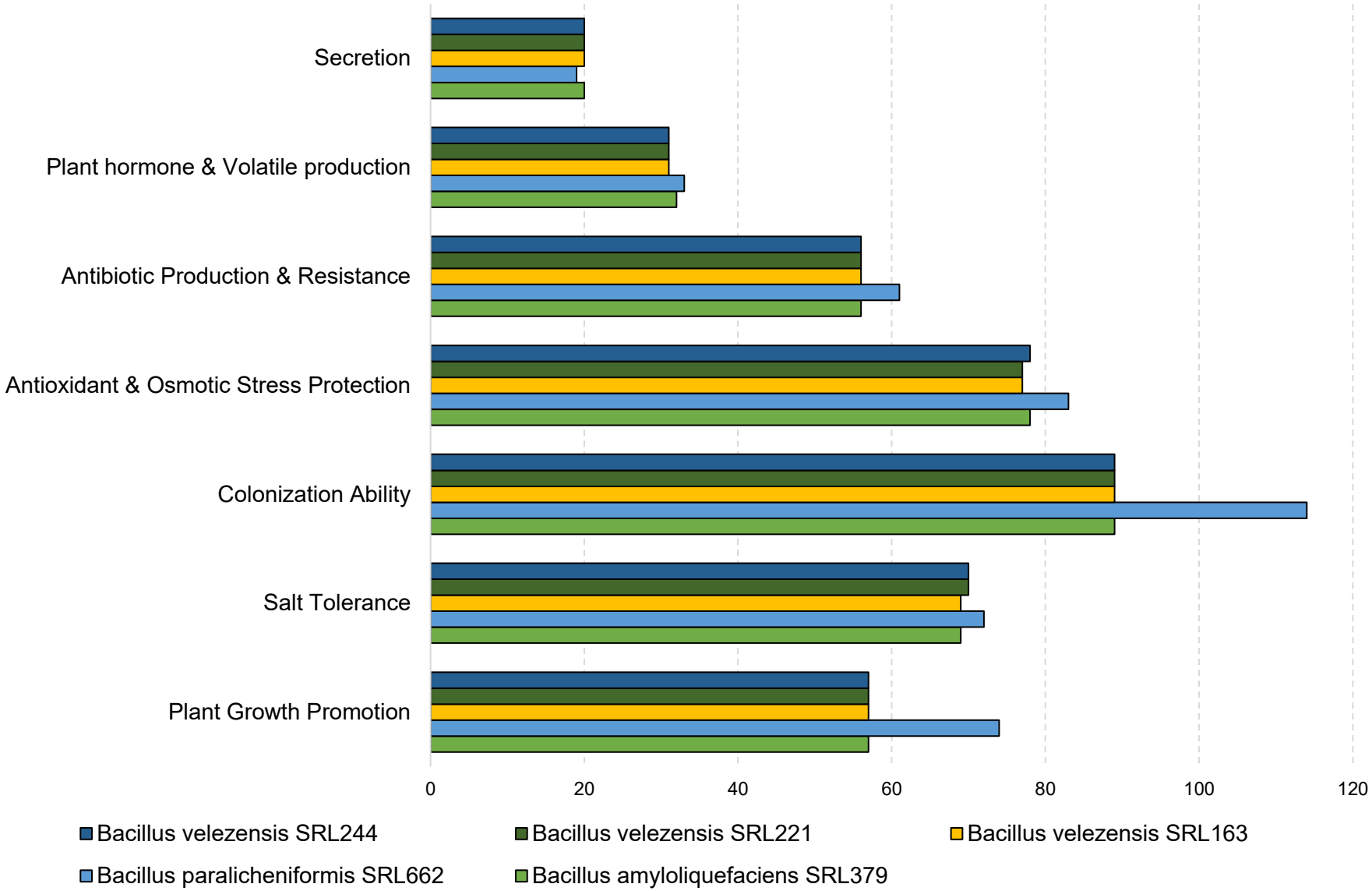


# Genome mining for plant-beneficial bacterial genes




**Dr Ch. Christakis**
former Post-Doc

# Comparative Genomics between our 25 isolates using Orthofinder

**Orthogroups provide information about**:

- The evolution of genes between species.
- The function of gene products (common origin is associated with common function)
- The **Genetic Novelty** within the group of the study

# Comparative Genomics between our 25 isolates using Orthofinder

## Genetic Novelty

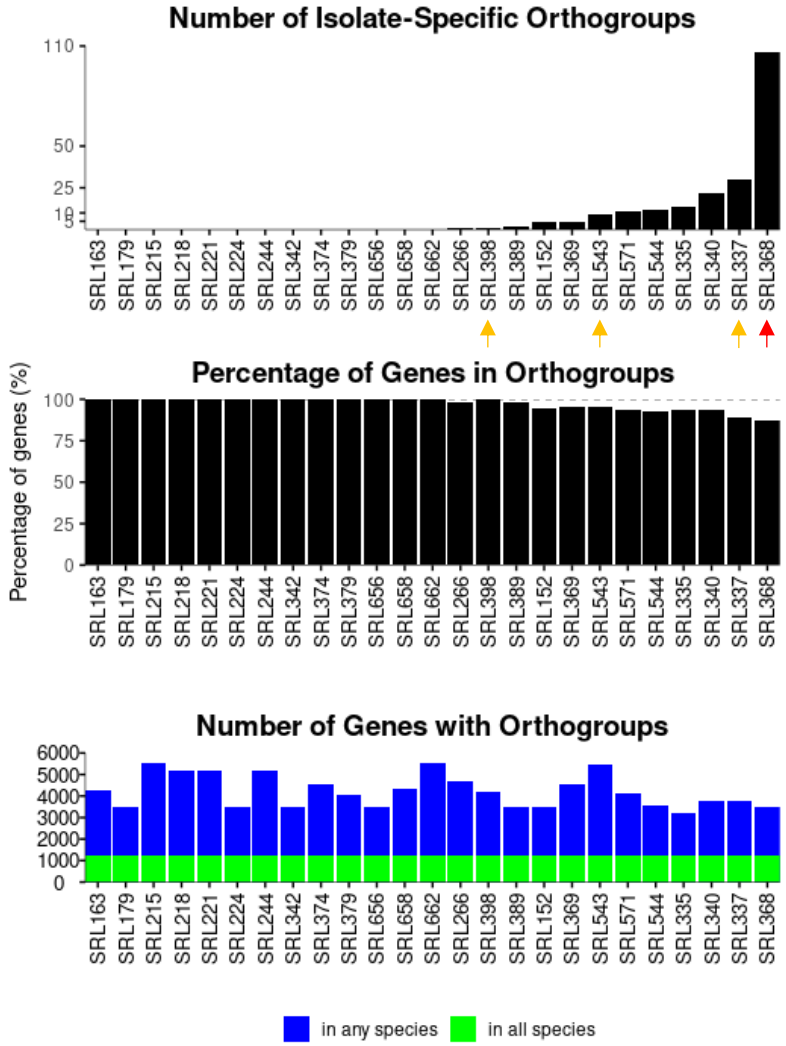
The **Genetic Novelty** refers to new elements (genes, gene groups, functions etc.) that are present in a new species/lineage but are absent in the ancestral species/lineage.

Genetic Novelty can be identified by detecting genes that:

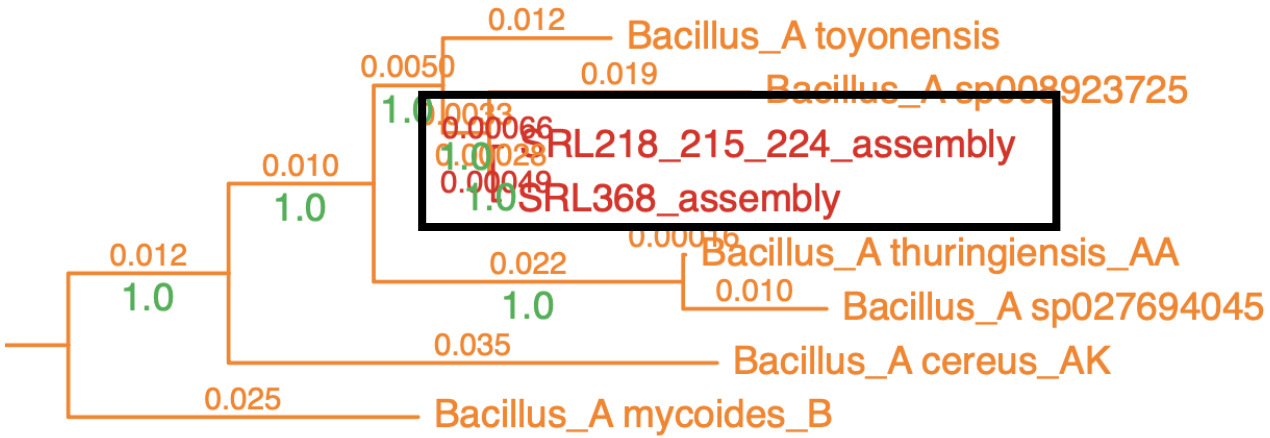
- **Do not belong to an Orthogroup**
- Appear in **a single species or a small number of closely related species** (putatively recent emergence)
- Appear in a **species-specific Orthogroup**

# Comparative Genomics between our 25 isolates using Orthofinder

## v) OrthoFinder results

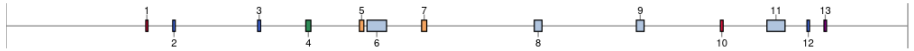



**<u>Putative novel species</u>: SRL398, SRL337, SRL342, SRL179, SRL543**



# antiSMASH result for the detection of biosynthetic gene clusters (BGCs)

## SRL398



| Region | Type | From | To | Most similar known cluster | | Similarity |
|---|---|---|---|---|---|---|
| Region 1.1 | NI-siderophore | 1,119,345 | 1,137,021 | | | |
| Region 1.2 | proteusin | 1,335,233 | 1,355,475 | | | |
| Region 1.3 | lassopeptide | 2,014,963 | 2,038,931 | paeninodin | RiPP | 60% |
| Region 1.4 | NRPS-like | 2,400,814 | 2,441,707 | | | |
| Region 1.5 | T3PKS | 2,830,061 | 2,865,828 | corynecin III/corynecin I/ corynecin II | Other | 13% |
| Region 1.6 | transAT-PKS , NRPS | 2,890,875 | 3,047,662 | bacitracin | NRP | 77% |
| Region 1.7 | T3PKS | 3,328,253 | 3,369,410 | | | |
| Region 1.8 | NRPS , transAT-PKS | 4,230,642 | 4,292,555 | paenilipoheptin | NRP+Polyketide | 23% |
| Region 1.9 | transAT-PKS , T1PKS , NRPS | 5,048,087 | 5,109,944 | pellasoren/pellasoren B | NRP+Polyketide:Modular type I polyketide | 33% |
| Region 1.10 | opine-like-metallophore | 5,721,144 | 5,743,296 | bacillopaline | Other | 100% |
| Region 1.11 | NRPS , transAT-PKS | 6,093,583 | 6,238,902 | polymyxin | NRP | 100% |
| Region 1.12 | cyclic-lactone-autoinducer | 6,414,479 | 6,435,018 | | | |
| Region 1.13 | terpene | 6,550,640 | 6,571,512 | carotenoid | Terpene | 33% |

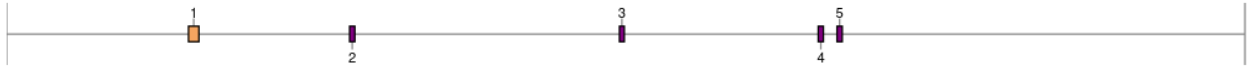
## SRL337

| Region | Type | From | To | Most similar known cluster | | Similarity |
|---|---|---|---|---|---|---|
| Region 1 | thiopeptide , LAP | 431,657 | 461,376 | | | |
| Region 2 | terpene | 2,792,361 | 2,814,232 | longicatenamide B/longicatenamide C/longicatenamide A/longicatenamide D | NRP | 7% |
| Region 3 | T3PKS | 2,928,543 | 2,969,616 | | | |

## SRL179

| Region | Type | From | To | Most similar known cluster | | Similarity |
|---|---|---|---|---|---|---|
| Region 1 | LAP | 1,076,885 | 1,100,431 | GG exopolysaccharide | Saccharide | 12% |
| Region 2 | betalactone | 2,350,294 | 2,382,428 | iturin | NRP+Polyketide | 22% |
| Region 3 | terpene | 2,896,647 | 2,912,796 | | | |
| Region 4 | T3PKS | 3,100,439 | 3,141,527 | | | |
| Region 5 | phosphonate | 4,387,028 | 4,402,411 | | | |
| Region 6 | terpene | 4,457,767 | 4,479,671 | | | |

## SRL543



| Region | Type | From | To | Most similar known cluster | | Similarity |
|---|---|---|---|---|---|---|
| Region 1.1 | T3PKS | 717,773 | 758,858 | | | |
| Region 1.2 | terpene | 1,353,423 | 1,374,124 | | | |
| Region 1.3 | terpene | 2,418,208 | 2,437,357 | carotenoid | Terpene | 50% |
| Region 1.4 | terpene | 3,202,382 | 3,222,871 | | | |
| Region 1.5 | terpene | 3,276,417 | 3,297,313 | | | |

# Total antiSMASH region counts for the 25 isolates

| Isolate ID | Species name | antiSMASH regions | regions with similarity ≤60% |
|---|---|---|---|
| SRL152 | *Peribacillus frigoritolerans* | 9 | 7 |
| SRL163 | *Bacillus velezensis* | 16 | 7 |
| SRL179 | *Neobacillus jeddahensis* | 6 | 6 |
| SRL215 | *Bacillus_A thuringiensis_S* | 16 | 14 |
| SRL218 | *Bacillus thuringiensis* | 16 | 14 |
| SRL221 | *Bacillus velezensis* | 16 | 7 |
| SRL224 | *Bacillus thuringiensis* | 16 | 14 |
| SRL244 | *Bacillus velezensis* | 16 | 7 |
| SRL266 | *Peribacillus frigoritolerans* | 9 | 6 |
| SRL335 | *Cytobacillus oceanisediminis* | 5 | 5 |
| SRL337 | *Bacillus salacetis* | 3 | 3 |
| SRL340 | *Peribacillus frigoritolerans* | 8 | 7 |
| SRL342 | *Paenibacillus sp001955855* | 13 | 10 |
| SRL368 | *Bacillus thuringiensis* | 15 | 12 |
| SRL369 | *Bacillus infantis* | 5 | 5 |
| SRL374 | *Bacillus velezensis* | 15 | 7 |
| SRL379 | *Bacillus velezensis* | 15 | 7 |
| SRL389 | *Peribacillus frigoritolerans* | 9 | 6 |
| SRL398 | *Paenibacillus sp001955855* | 13 | 10 |
| SRL543 | *Bacillus infantis* | 5 | 5 |
| SRL544 | *Rossellomorea haikouensis* | 5 | 5 |
| SRL571 | *Bacillus altitudinis* | 12 | 9 |
| SRL656 | *Bacillus paralicheniformis* | 11 | 8 |
| SRL658 | *Bacillus paralicheniformis* | 11 | 8 |
| SRL662 | *Bacillus paralicheniformis* | 18 | 16 |

# Future plans

- Study of genes that are not included in Orthogroups

- Comparison of selected isolates with their **closest relatives** (**pangenome analysis**).

# Conclusions

1) The **biodiversity of the endophytic microbiome** is profound and can be used to identify valuable **beneficial microbes**

**2) Four putative novel species** of Bacilli endophytes have been isolated and cultivated

**3) Increased genetic, functional and taxonomic novelty** among our new species and even in our isolates of already known species

4) The **deep exploration of the endophytic microbial diversity** is a key for advances in microbiology, ecology and agriculture

# Our team and collaborators!

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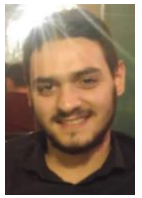
former Post-Doc

**Mr M. Avramakis**
Botanist

ΕΛΓΟ-ΔΗΜΗΤΡΑ
ΕΛΛΗΝΙΚΟΣ ΓΕΩΡΓΙΚΟΣ ΟΡΓΑΝΙΣΜΟΣ - ΔΗΜΗΤΡΑ

HELLENIC MEDITERRANEAN UNIVERSITY



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SARRIS-LAB

BIOCONTROL
Microbiome & Metabolites in Agriculture

# Thank you!

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