Microbial genomics and its application to biocontrol research; current and future perspectives

BPIA Meeting 27 March 2024

Crop Bioprotection Research Unit

Christopher Dunlap

National Center for Agricultural Utilization Research, Peoria IL
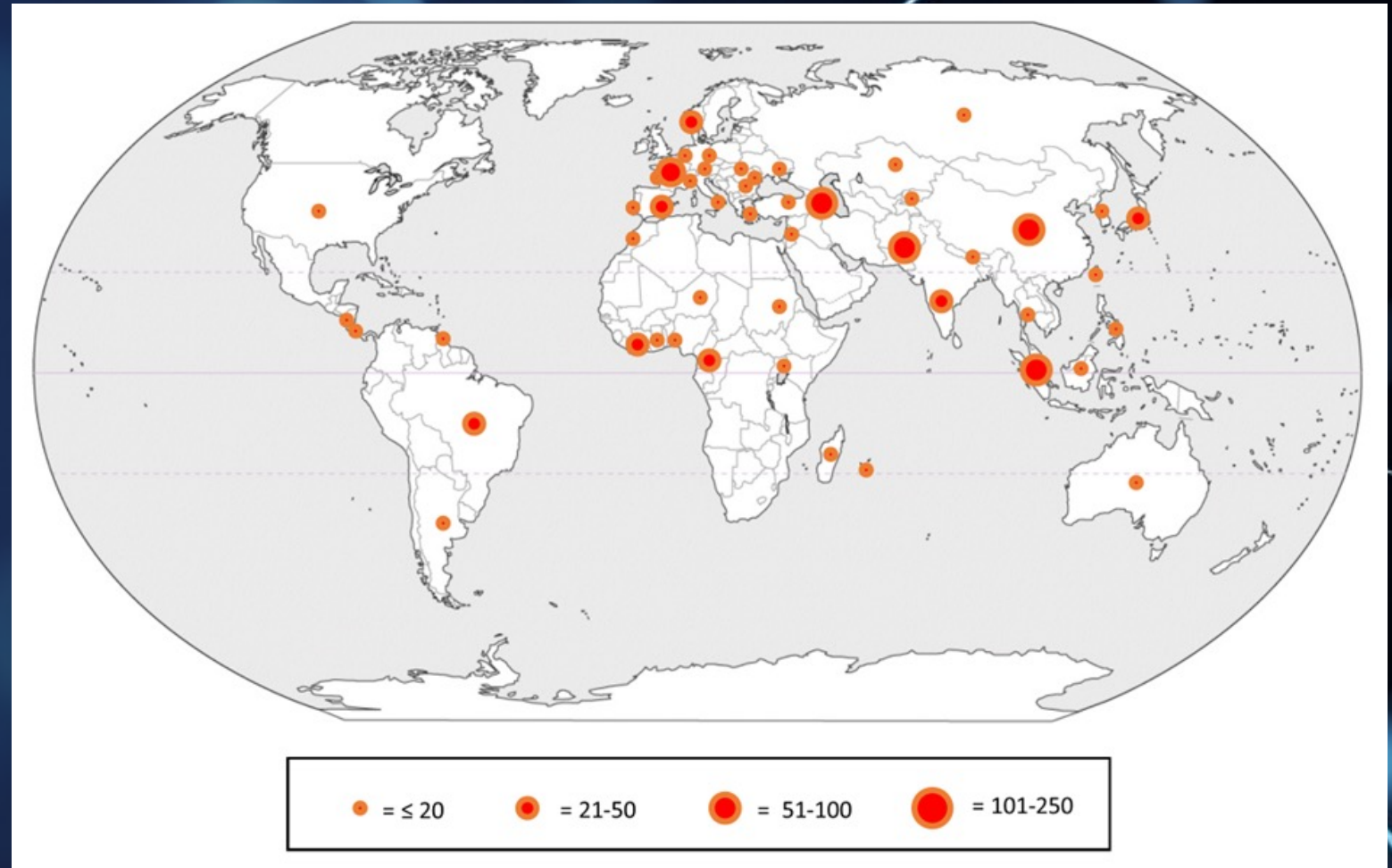
Christopher.Dunlap@usda.gov

Current Research Projects

- Genomics of entomopathogenic fungi (-023)

- Genomics of biocontrol bacteria (-024)

- Genomics of bee associated microbes (-023, beenome)

- Biocontrol of ambrosia beetles (-024 NPDRS)

Genomics of entomopathogenic fungi

- Based on collection of ~1000 strains inherited from EBCL.

- Draft genomes for all and completed genomes for key species.

- Sequencing complete, working on data analysis and archiving.

- CRADA with a drug discovery company.

= ≤ 20   = 21-50   = 51-100   = 101-250

Genomics of entomopathogenic fungi

- The large data set offers us insights into the role of secondary metabolites (NRPS/PKS gene clusters) in EPF.

Species specific clusters — Shared clusters

Genomics of entomopathogenic fungi

Understanding epigenetic control of gene expression in fungi

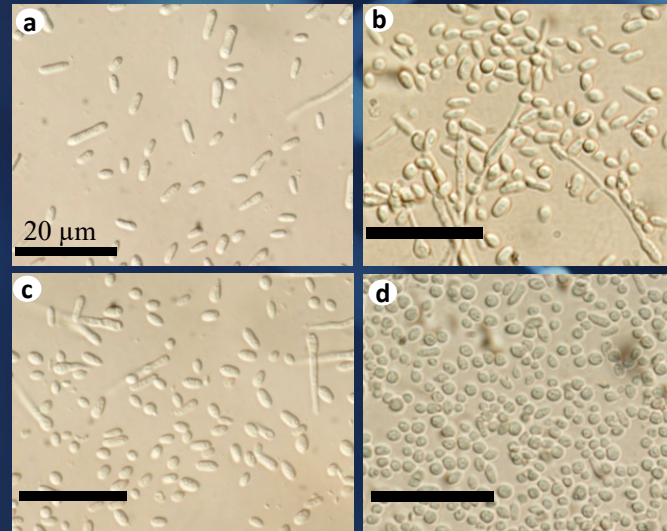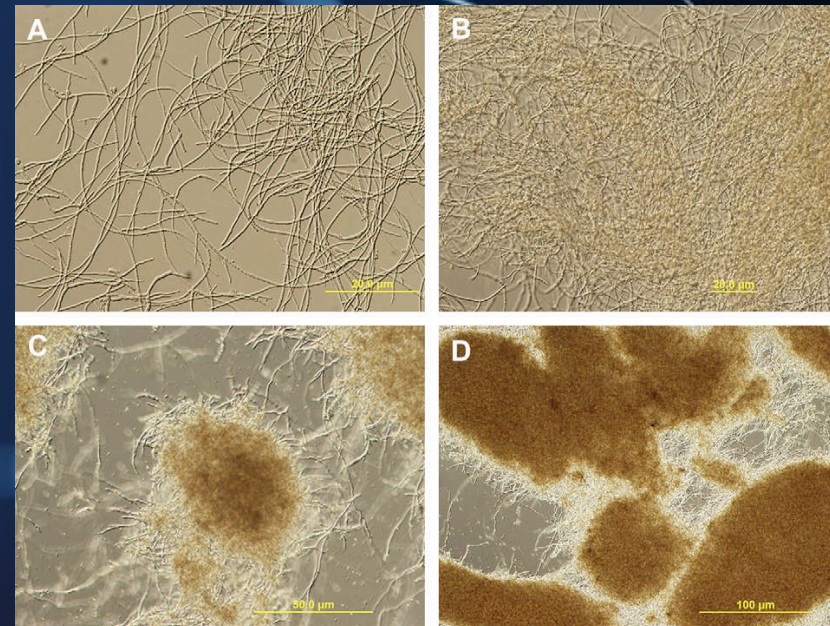- Phase II is using Hi-C sequencing to associate chromatin status with key lifestyle events
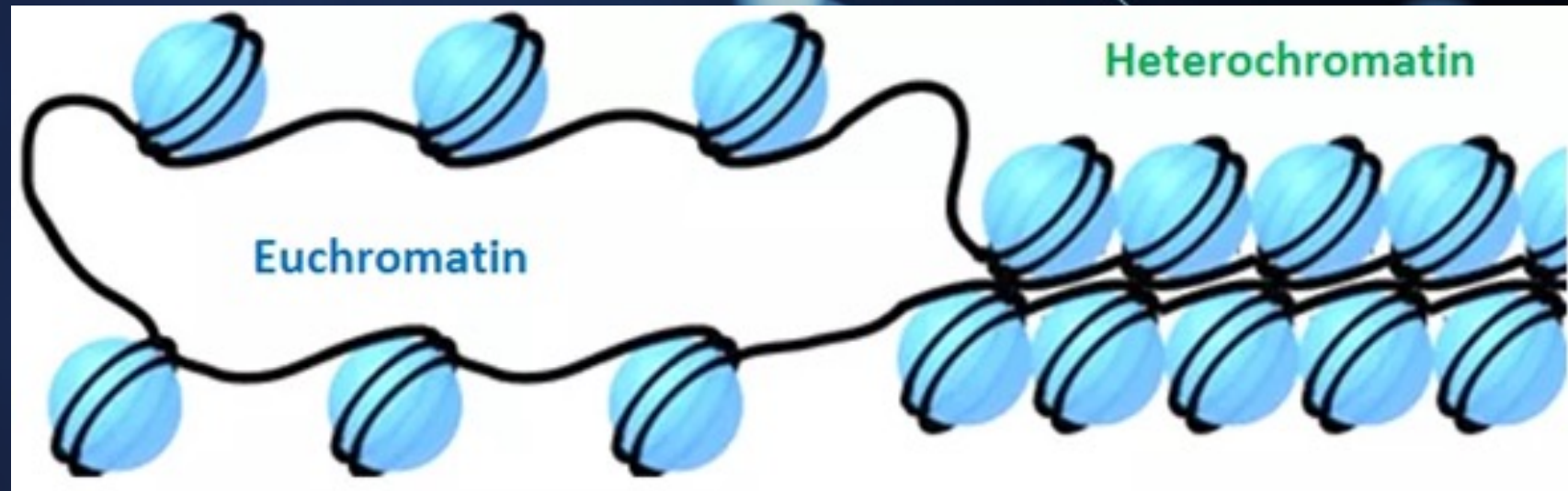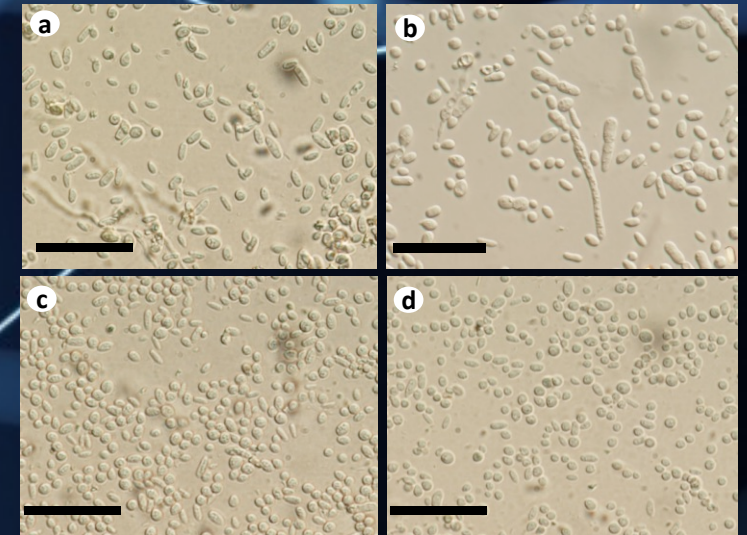
Genomics of entomopathogenic fungi

- Why is understanding epigenetics important for EPF?

- Associated with morphology transitions, a long-term goal of controlling which morphology we produce.
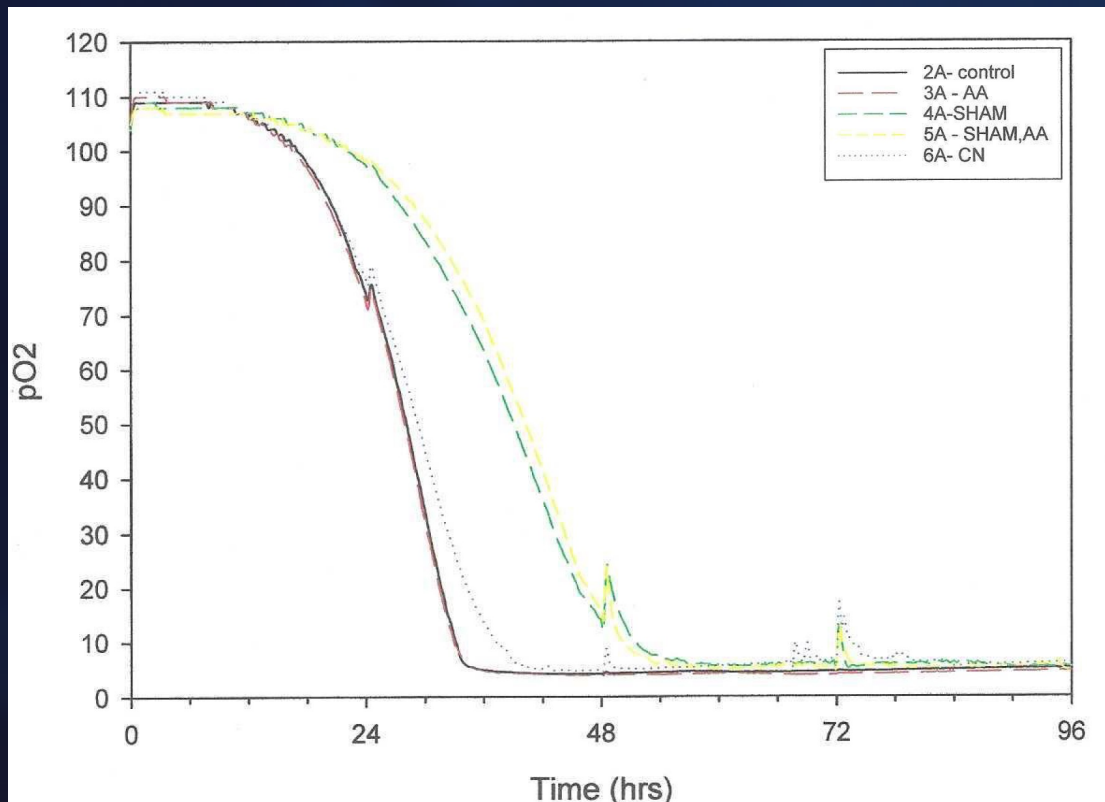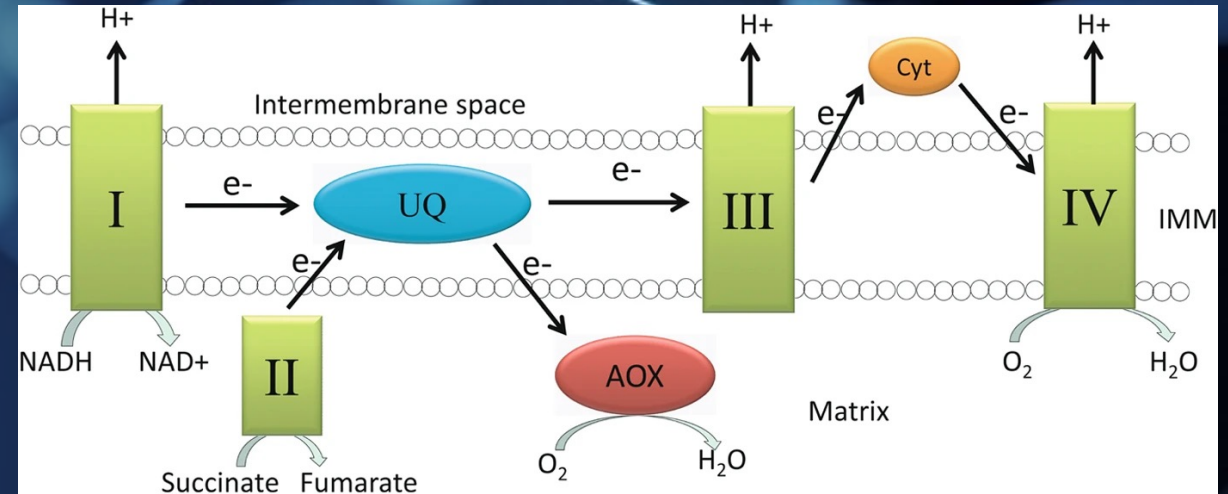

C) ESALQ1432

D) GHA

Genomics of entomopathogenic fungi

Fungal respiration during the production of entomopathogenic fungi

Fungal respiration in the presence of inhibitors for *Beauveria bassiana*.

Genomics of biocontol bacteria

- Collaboration between the ARS Culture Collection (NRRL)

- Goal is to sequence ~5000 Bacillus sp strains.

- Collaboration with Dr Fred Cohan of Wesleyan University on Bacillus evolution

Genomics of biocontol bacteria

- Post-genomic project

- Identify and express unknown biosynthetic clusters.

- Identify new metabolites for crop protection applications.

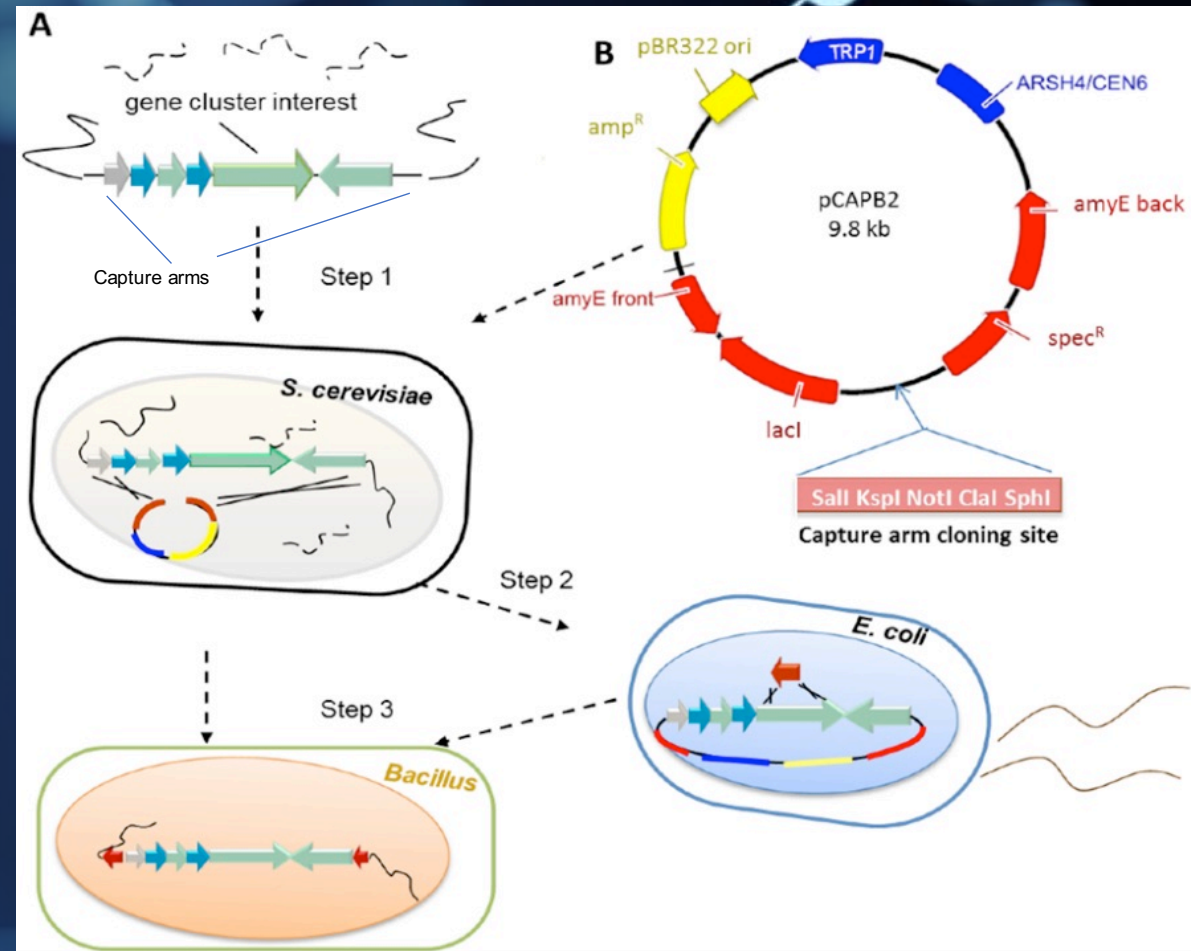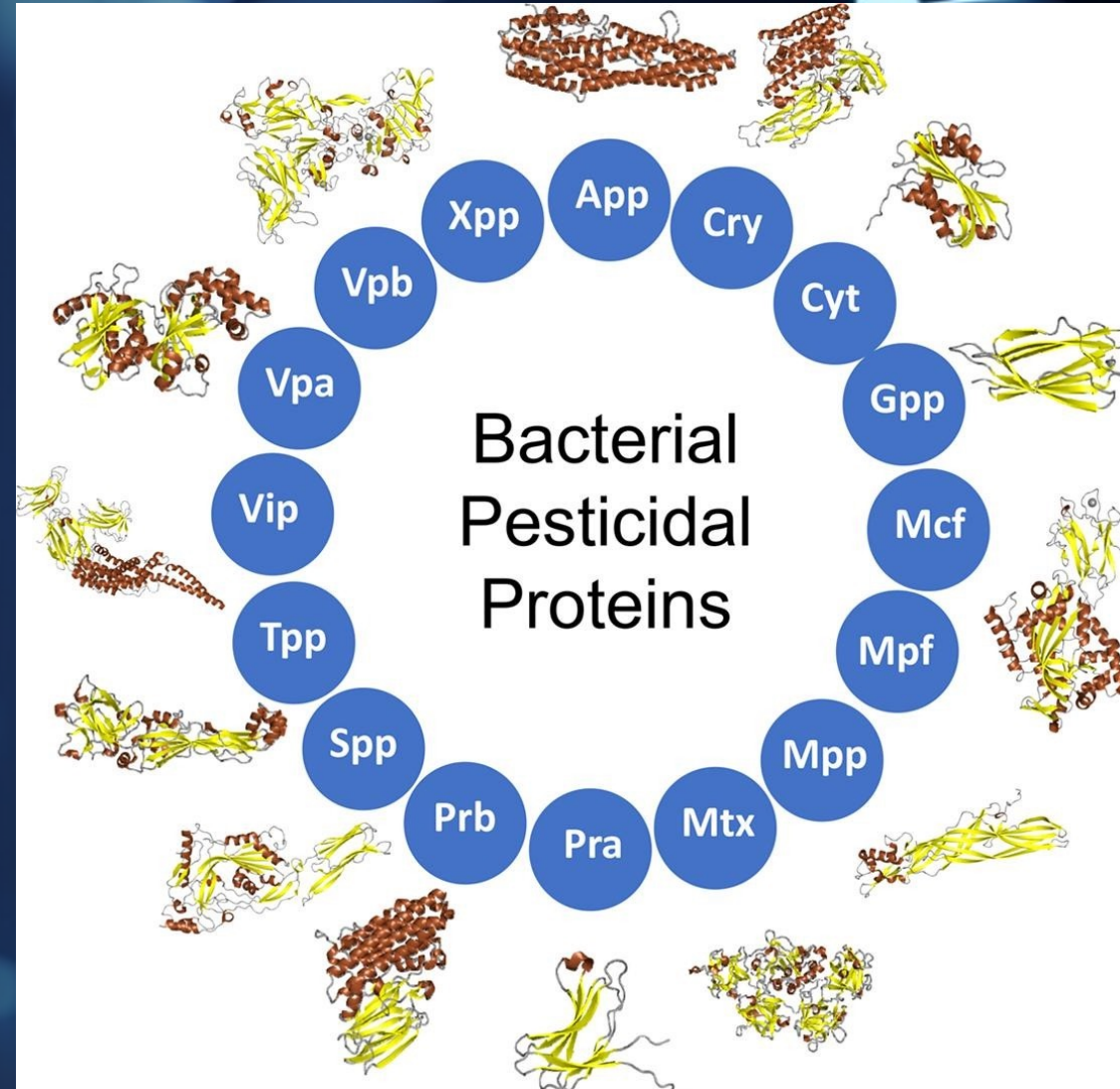- Other functional genomics experiments.

Figure 2. Schematic of a TAR-based cloning and expression system, Taken from Li et al. 2015.
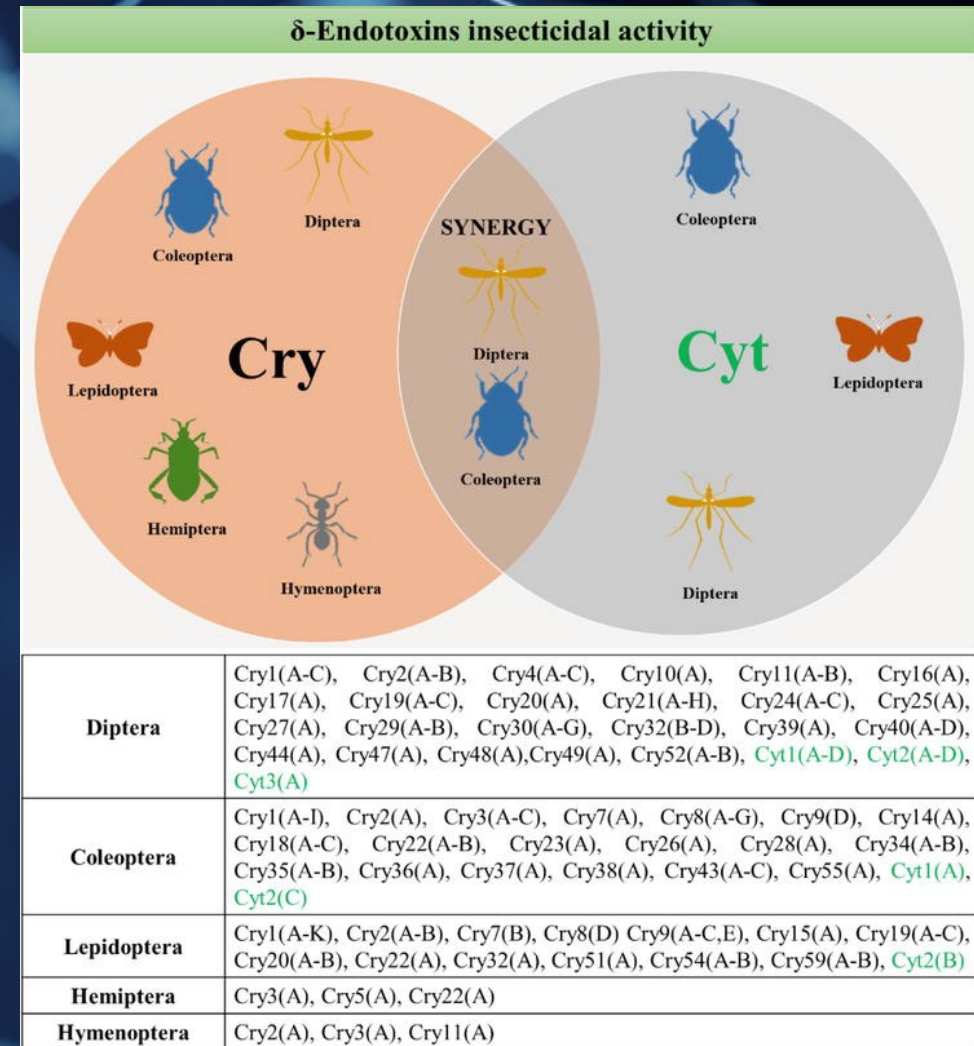
Genomics of biocontol bacteria

- Genome mining for new insect toxins

- Examined more than 5,500 genomes so far.
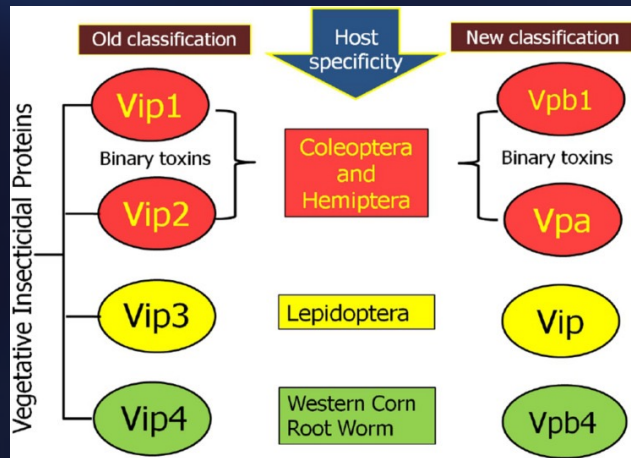
- 12,607 putative BIPs identified.

Genomics of biocontol bacteria

- Cry toxins 6381 identified.

- 227 below 44% AA homology

- 488 Between 44-75% AA homology

- Cyt toxins 123 identified.

- 18 below 44% AA homology

- 4 Between 44-75% AA homology

δ-Endotoxins insecticidal activity

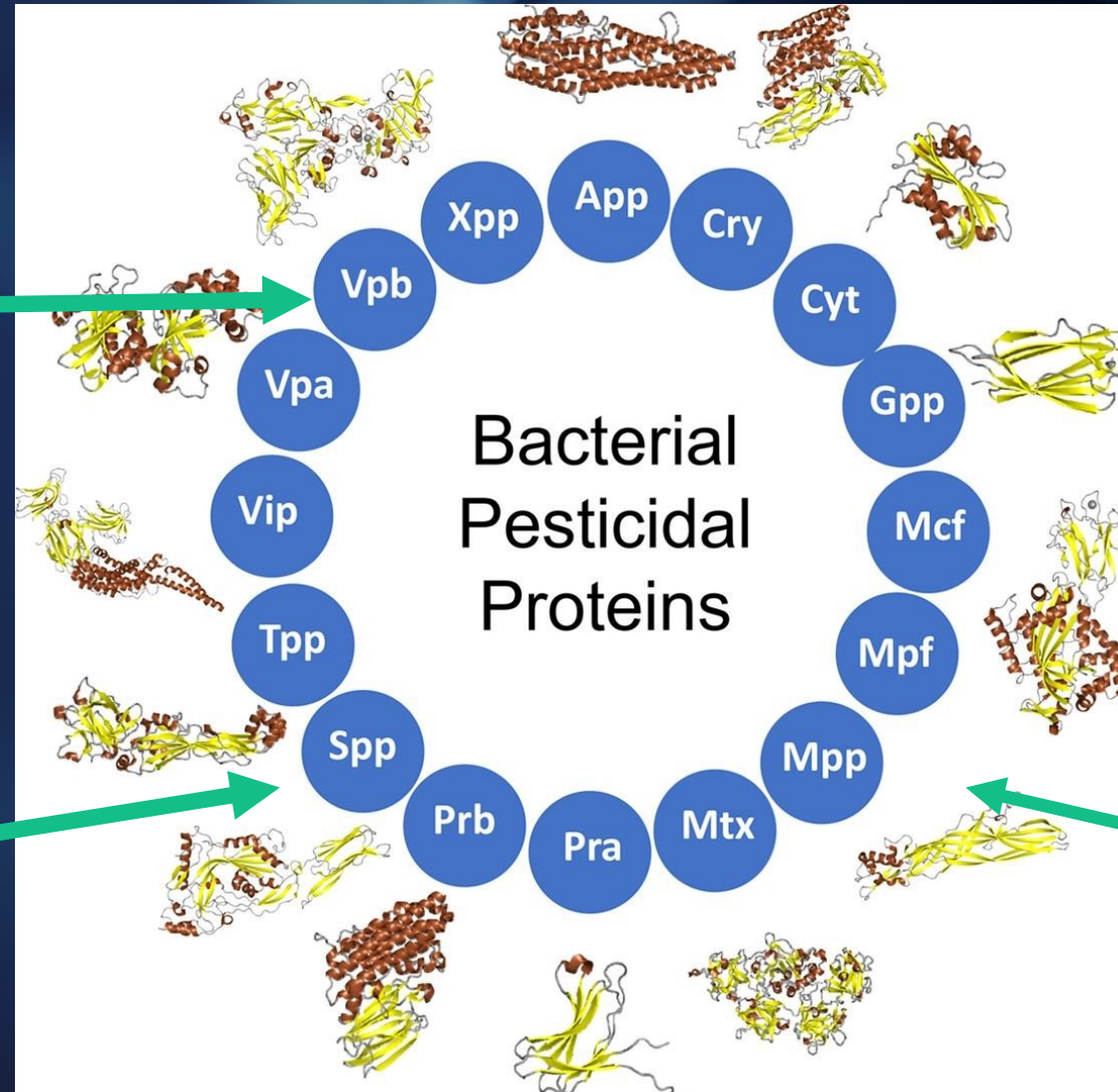|	
Diptera	Cry1(A-C), Cry2(A-B), Cry4(A-C), Cry10(A), Cry11(A-B), Cry16(A), Cry17(A), Cry19(A-C), Cry20(A), Cry21(A-H), Cry24(A-C), Cry25(A), Cry27(A), Cry29(A-B), Cry30(A-G), Cry32(B-D), Cry39(A), Cry40(A-D), Cry44(A), Cry47(A), Cry48(A),Cry49(A), Cry52(A-B), Cyt1(A-D), Cyt2(A-D), Cyt3(A)
Coleoptera	Cry1(A-I), Cry2(A), Cry3(A-C), Cry7(A), Cry8(A-G), Cry9(D), Cry14(A), Cry18(A-C), Cry22(A-B), Cry23(A), Cry26(A), Cry28(A), Cry34(A-B), Cry35(A-B), Cry36(A), Cry37(A), Cry38(A), Cry43(A-C), Cry55(A), Cyt1(A), Cyt2(C)
Lepidoptera	Cry1(A-K), Cry2(A-B), Cry7(B), Cry8(D) Cry9(A-C,E), Cry15(A), Cry19(A-C), Cry20(A-B), Cry22(A), Cry32(A), Cry51(A), Cry54(A-B), Cry59(A-B), Cyt2(B)
Hemiptera	Cry3(A), Cry5(A), Cry22(A)
Hymenoptera	Cry2(A), Cry3(A), Cry11(A)

Genomics of biocontol bacteria
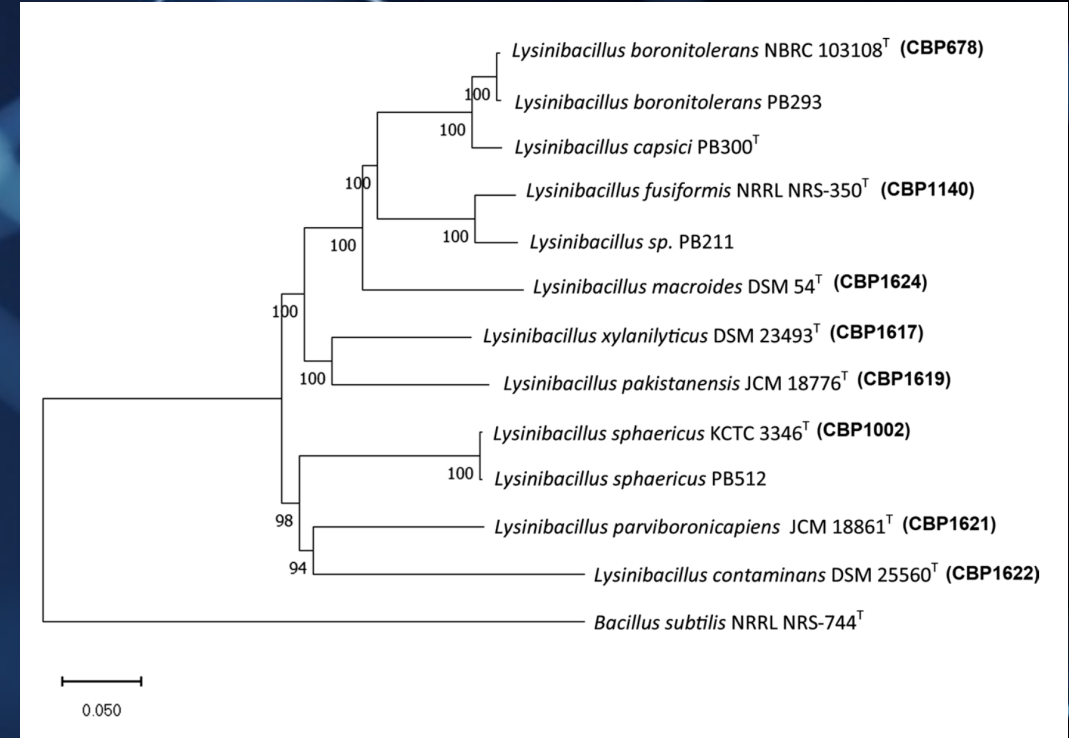

1280

2057

1157

Spp = sphaericolysin like toxins

Mpp formerly MTX2, MTX3 and other Cry

Lysinibacillus spp. as plant growth promters

Bacillus taxonomy

- One of the reasons that you may hate me.

- Before genomics, Bacillus taxonomy was incredibly hard and stupid.

- I have serendipitously become an expert in *Bacillus* taxonomy.

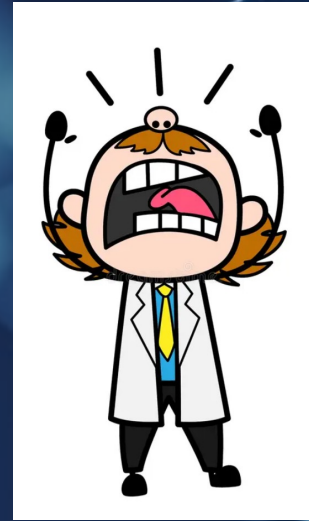- In 2011, the first strain I sequenced was a *Bacillus* strain.
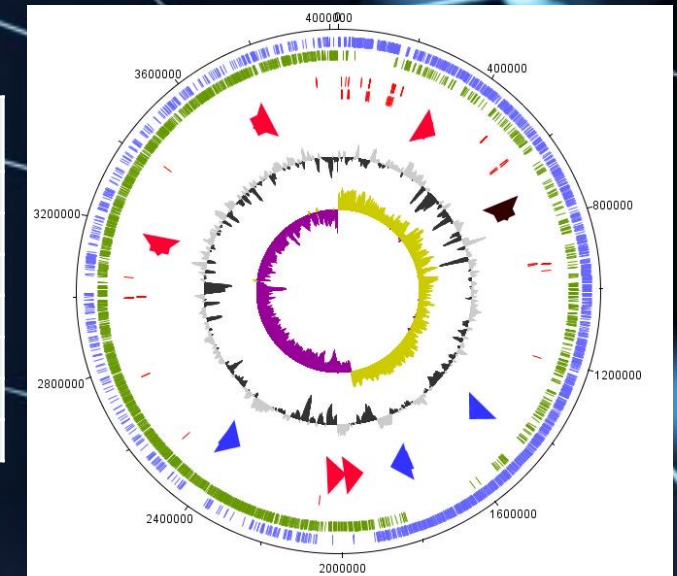
| Table 1. Genome statistics of B. amyloliquefaciens AS 43.3	
size	3961291 bp
Number of genes	4037
Number of proteins	3919
tRNAs	89
rRNAs	29
GC%	46.60%
average coverage	43x

Bacillus taxonomy

- Cheonggukjang is a fermented soybean paste similar Japanese natto. These are some of the richest food sources for vitamin K. These fermentations are dominated by *Bacillus* spp.

- At this time, we were still missing many reference genomes.
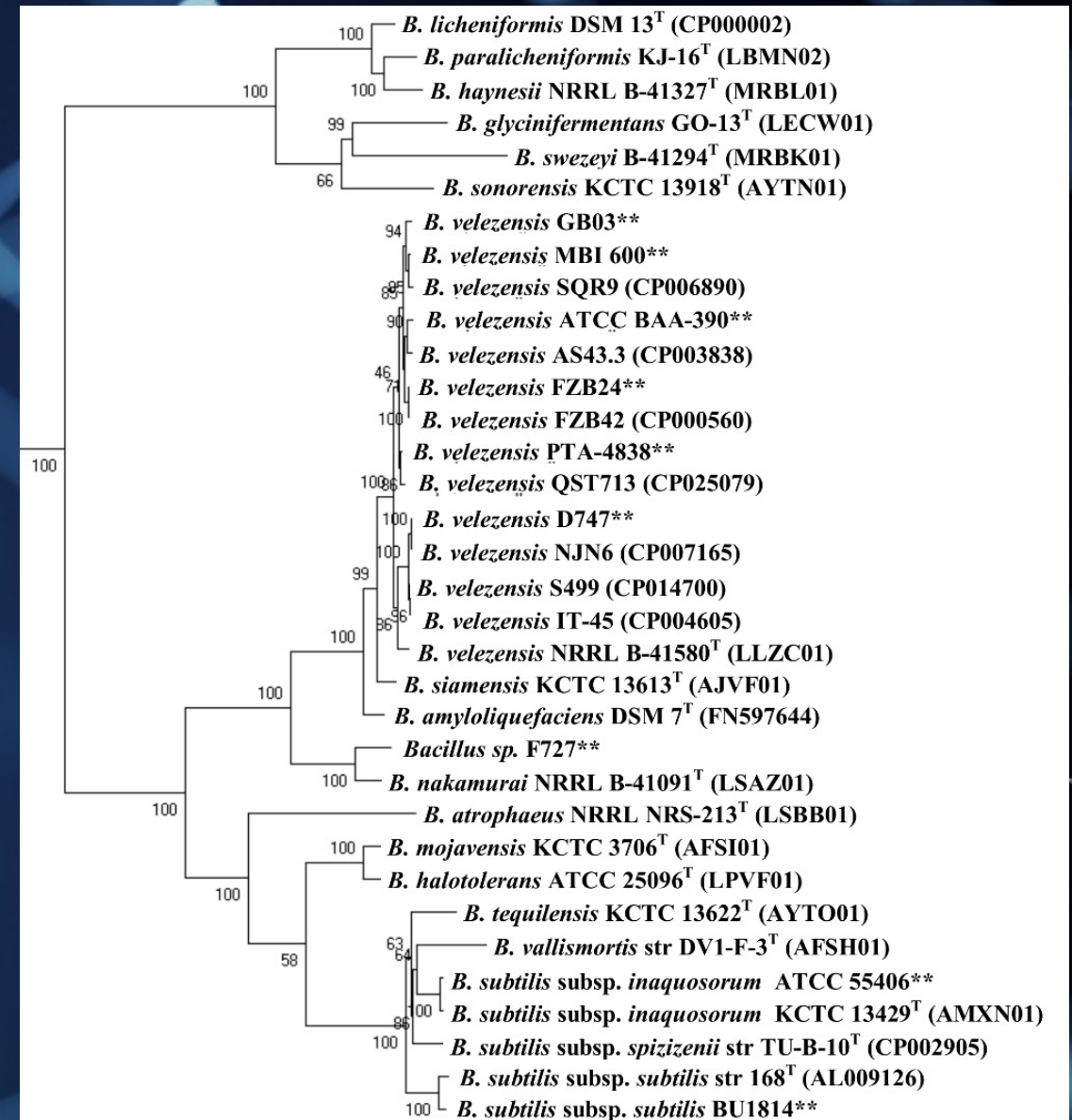
Bacillus taxonomy

The taxon so popular it was described four times
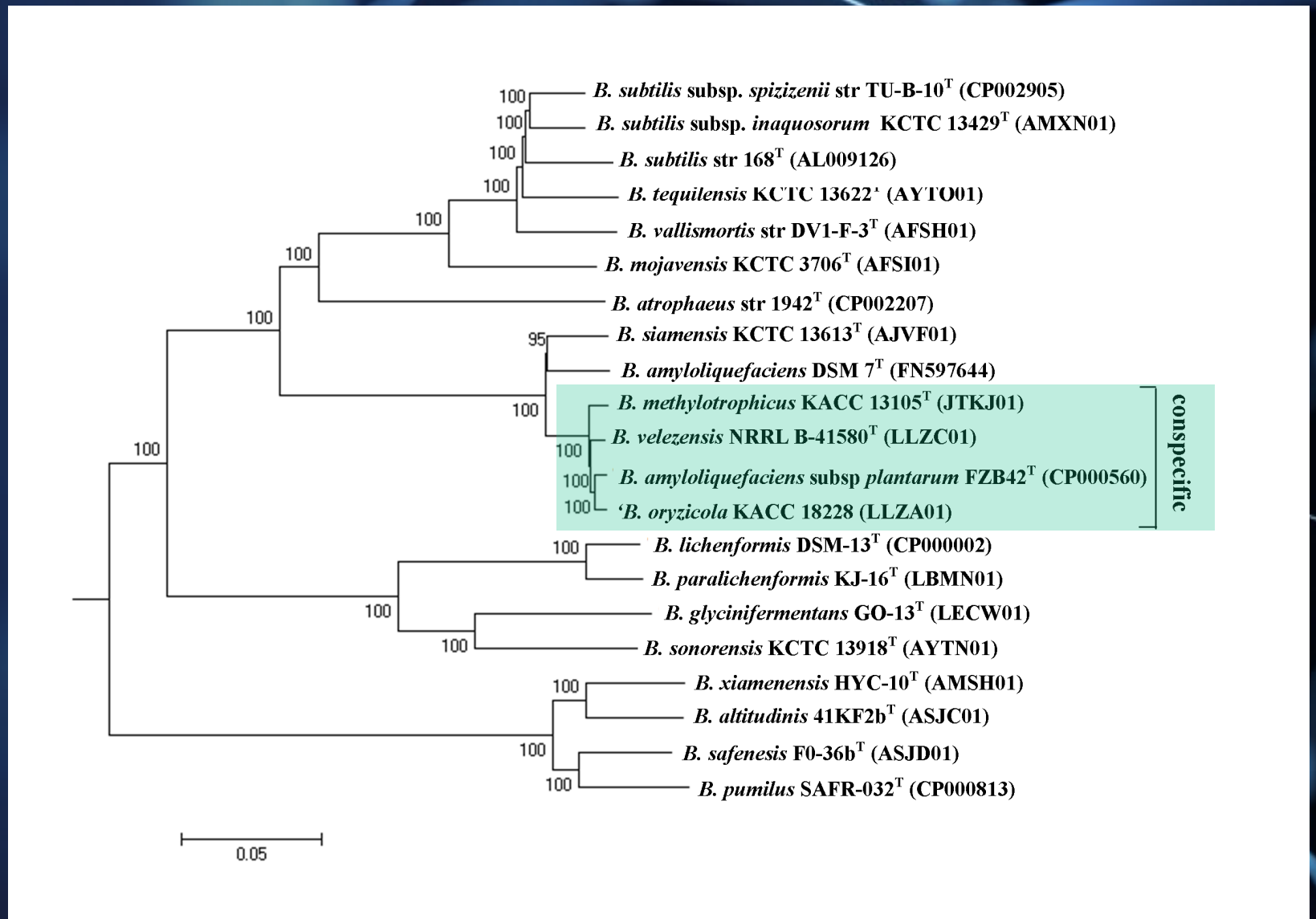
Bacillus taxonomy

- Most of the strains registered as *Bacillus* plant pathogen antagonists are *B. velezensis*. There are probably more than 100 commercial products around the world containing this species, under various species nomenclature going back to 1971.

- Strains of *B. velezensis* are isolated so commonly that I developed *B. velezensis* specific primers for a simple amplicon assay.
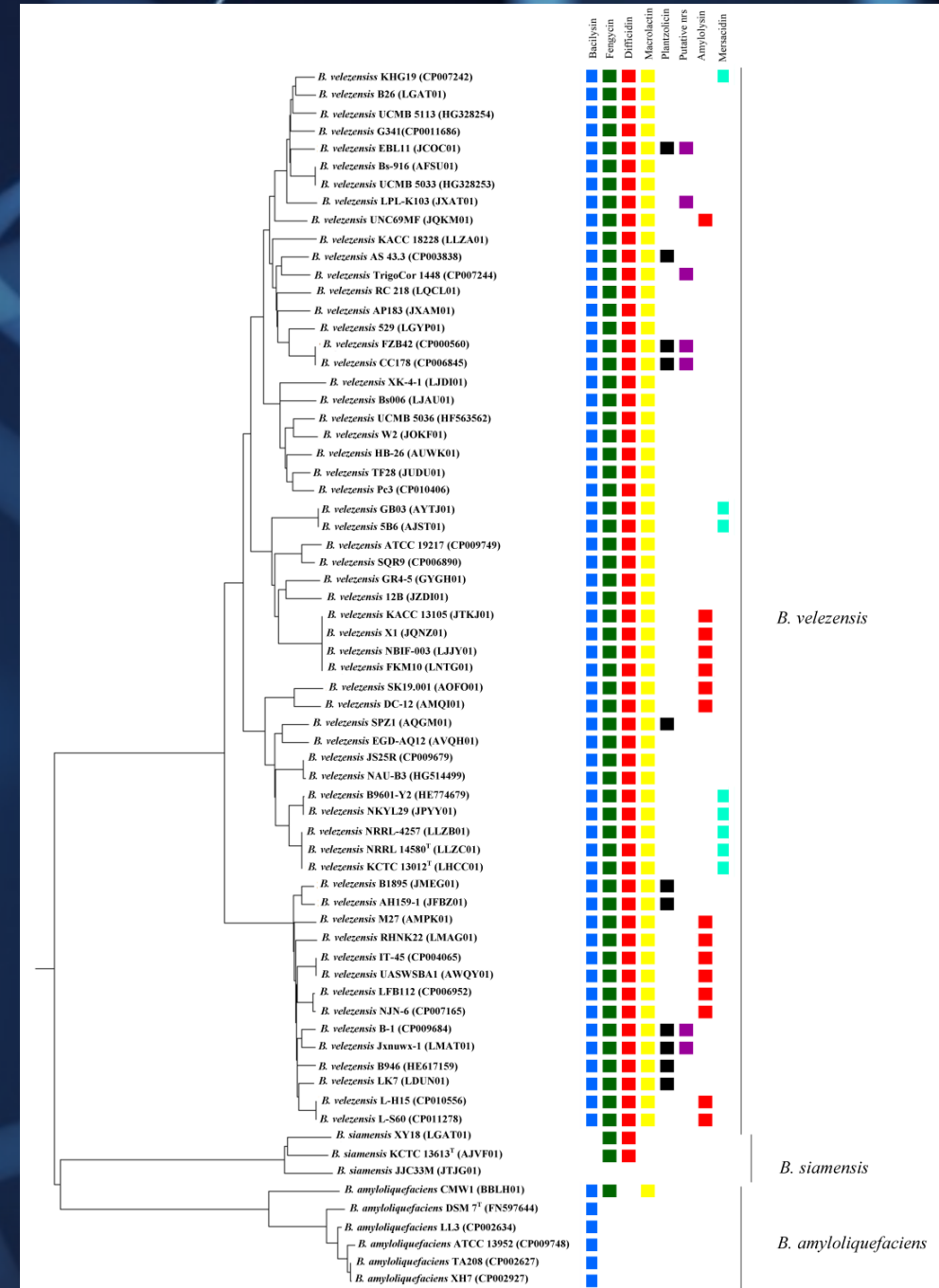

Dunlap, C.A., (2019). Taxonomy of registered *Bacillus* spp. strains used as plant pathogen antagonists. Biol. Control 134, 82-86.

Bacillus velezensis

- Why is *B. velezensis* a biocontrol super star and its near neighbors are not?

Compound	Synthetase type	Size (kb)	Bioactivity
Surfactin	NRPS	26.2	multiple
Iturin	NRPS	37.2	antifungal
Fengycin	NRPS	37.7	antifungal
Bacillibactin	NRPS	12.7	siderophore
Bacilysin/chlorotetaine	NRPS	6.7	antibacterial
Macrolactin	PKS	53.2	antibacterial
Bacillaene	PKS	72,5	antibacterial
Difficidin	PKS	69,5	antibacterial
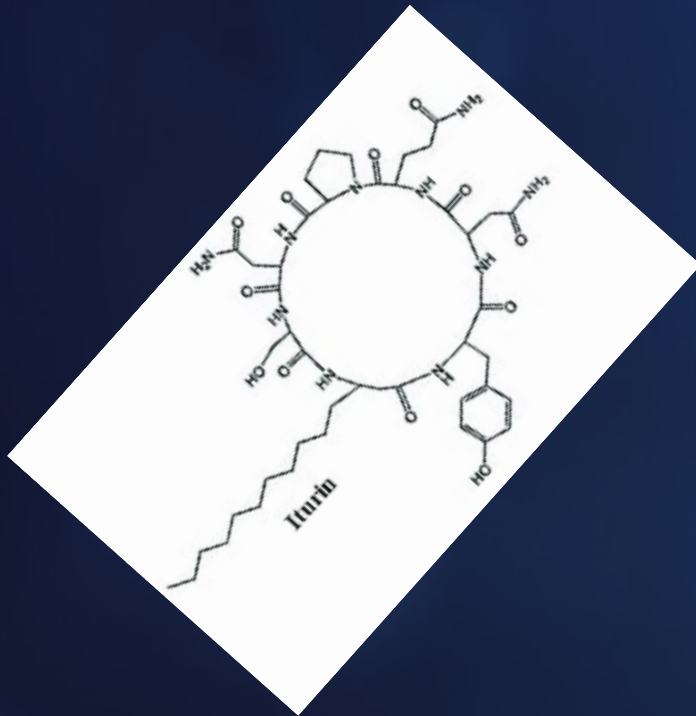
Iturins

Iturinic gene cluster organization
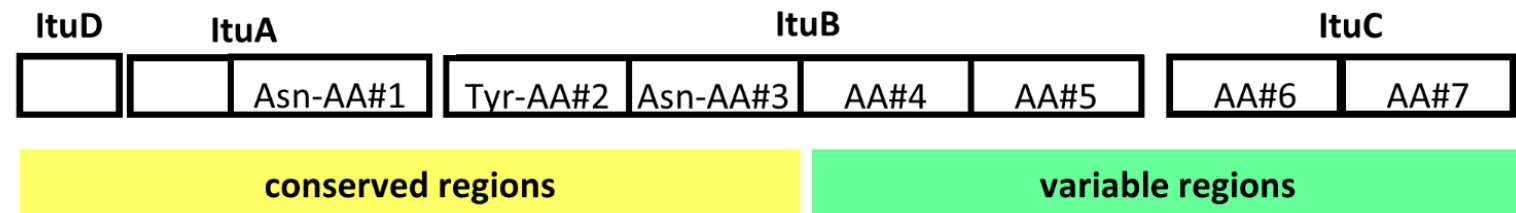
ItuD	ItuA		ItuB				ItuC	
		Asn-AA#1	Tyr-AA#2	Asn-AA#3	AA#4	AA#5	AA#6	AA#7

conserved regions | variable regions

Compound	AA#4	AA#5	AA#6	AA#7
Iturin A	Gln	Pro	Asn	Ser
Mycosubtilin	Gln	Pro	Ser	Asn
Bacillomycin L	Ser	Glu	Ser	Thr
Bacillomycin D	Pro	Glu	Ser	Thr
Bacillomycin F	Gln	Pro	Asn	Thr
Mojavensin	Gln	Pro	Asn	Asn

Moisset

Iturin diversity

- Usually all or none.

- Some are species specific.

- Several new producers identified.

Iturinic gene cluster organization

ItuD	ItuA		ItuB				ItuC	
		Asn-AA#1	Tyr-AA#2	Asn-AA#3	AA#4	AA#5	AA#6	AA#7

| conserved regions | | | | variable regions | | | | |

| Compound	AA#4	AA#5	AA#6	AA#7
Iturin A	Gln	Pro	Asn	Ser
Mycosubtilin	Gln	Pro	Ser	Asn
Bacillomycin L	Ser	Glu	Ser	Thr
Bacillomycin D	Pro	Glu	Ser	Thr
Bacillomycin F	Gln	Pro	Asn	Thr
Mojavensin	Gln	Pro	Asn	Asn

Bacillus evoluton

- Species evolution in *Bacillus* is highly dependent on metabolites and microbe-microbe interaction.

- Species differ by ~200 genes from nearest neighbor.

- Understanding these interactions will help us understand improve *Bacillus* consortium
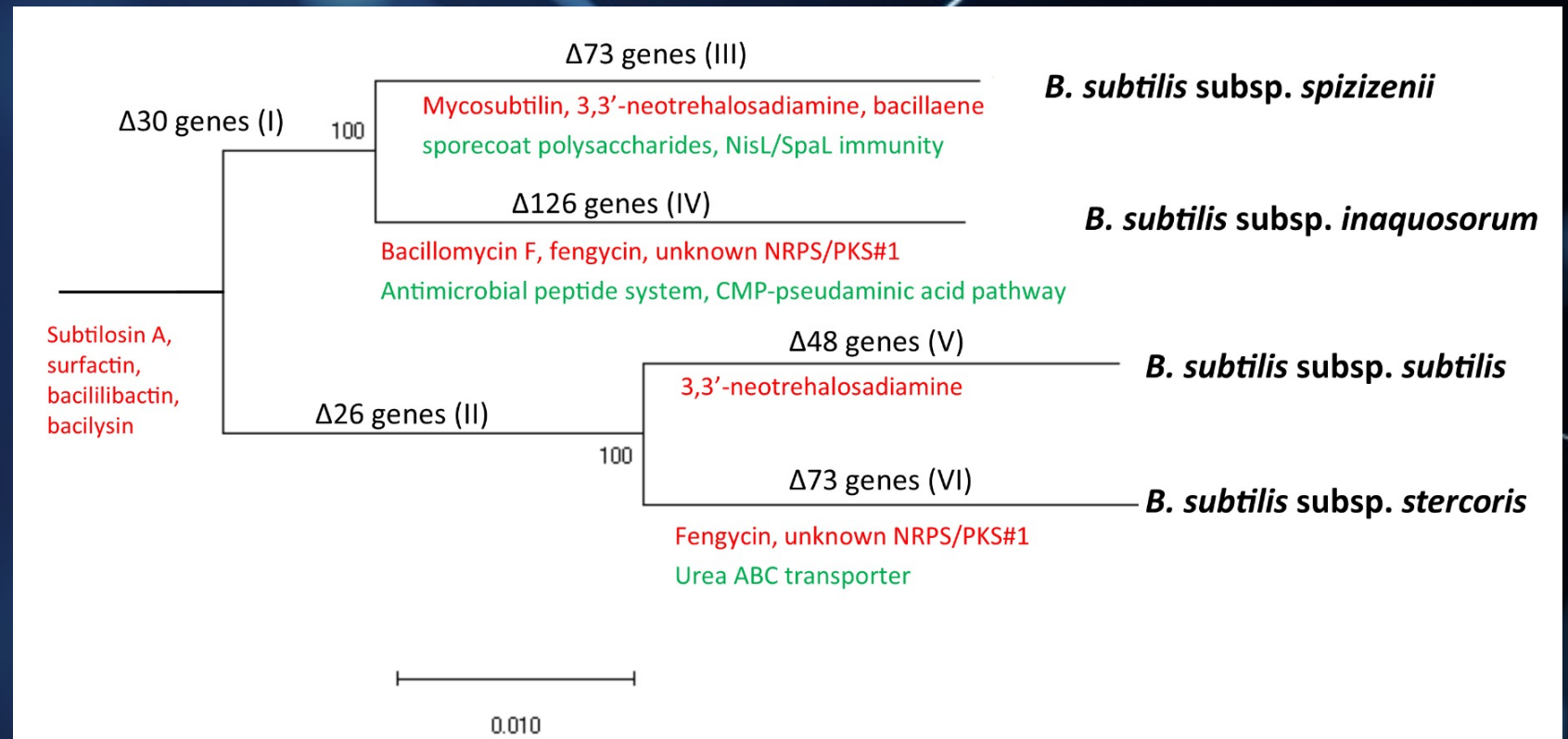
Other related projects

- NPDRS project on ambrosia beetles, vectoring with mites.

- Other NGS project developing primers for specialized community analysis (Bacillus, Pseudomonas, EPF, etc).
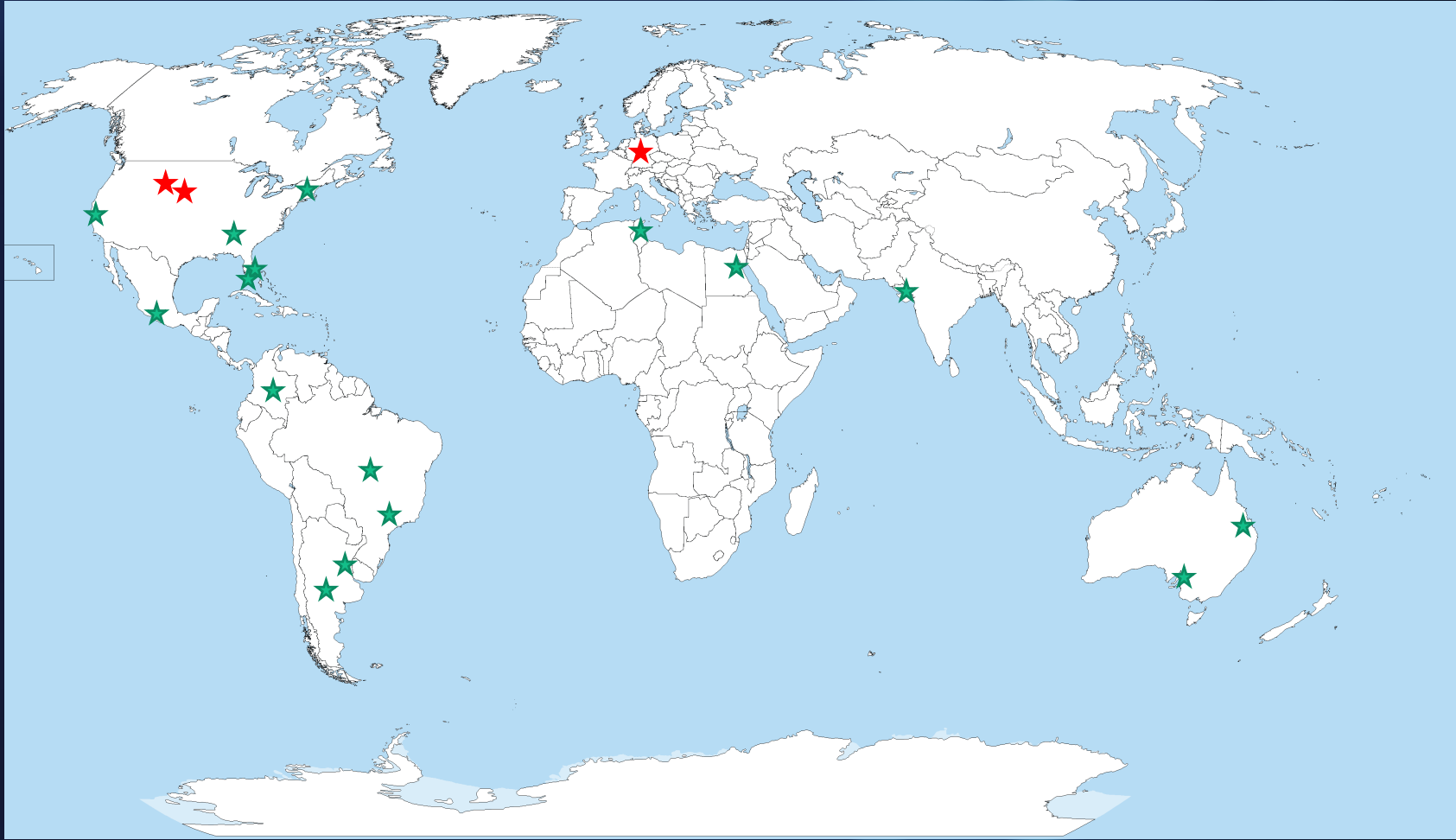

UGA2109039

bee projects

- Started last year – identified bee-associated microbes in NRRL and sequenced the first group ~80 strains (*Paenibacillus larvae, Paenibacillus apiarius*, etc).

- New post-doc to work on gut microbiome formation.

- Exploring e-DNA applications.

Photo by B. Moisset

Collaborations

Future directions

- The genomics era is ending or at least becoming commoditized.
- We are running out of things to sequence.
- Need value from these existing genomes.
- Genome mining and expression of novel metabolites.
- Functional genomics to understand the effect of genotype.
- Transformable environmental strains.
- GMO strains for a variety of applications.
- Continued evolution of eDNA applications.
- New sequencing techniques (e.g. Hi-C, Tn-Seq, etc).
- New microbiome techniques.

What are BPIA research needs?

- Are there specific research needs for the industry?
- ARS is better suited to address some needs than University Scientists, since some areas aren't open to grant funding.
- Taxonomy, generate data to support regulatory issues, and other less flashy research.
- We are also able to do company specific research if it is the right fit.

Acknowledgements

None of this would be possible without great technical staff.

Heather

Ben

Angela

Maddy