



**BBSRC-FAPESP JOINT PUMP-PRIMING AWARDS for AMR
and INSECT PEST RESISTANCE IN AGRICULTURE:
*Understanding and managing resistance, including novel
methods, for pathogen and pest control.***

PARTNERSHIP BUILDING WORKSHOP

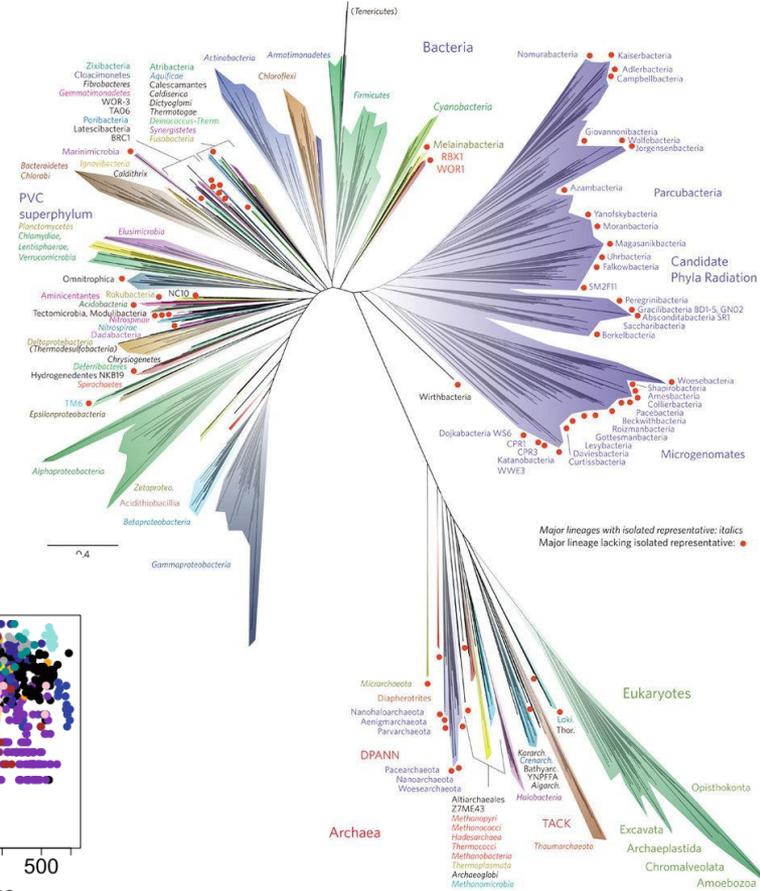
Tsai Siu Mui, Prof.
tsai@cena.usp.br

Cell and Molecular Laboratory
Center for Nuclear Energy in Agriculture
University of São Paulo

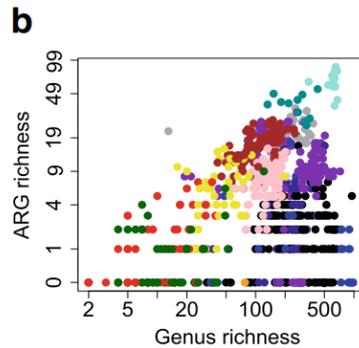
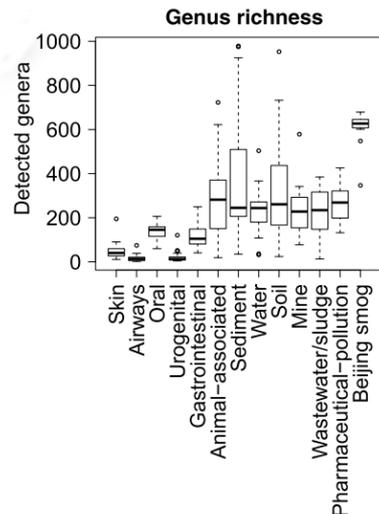
5-6 September, 2017
São Paulo - BR

→ Most microbes do not grow using traditional cultivation methods and hence are referred to as “*unculturable*”.

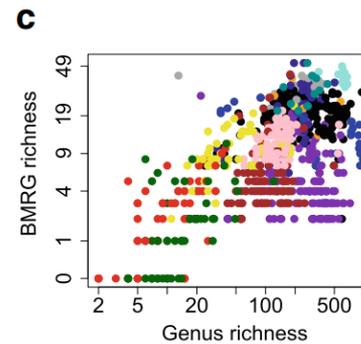
→ Antibiotic Resistance Genes (ARG) in *unculturable* bacteria



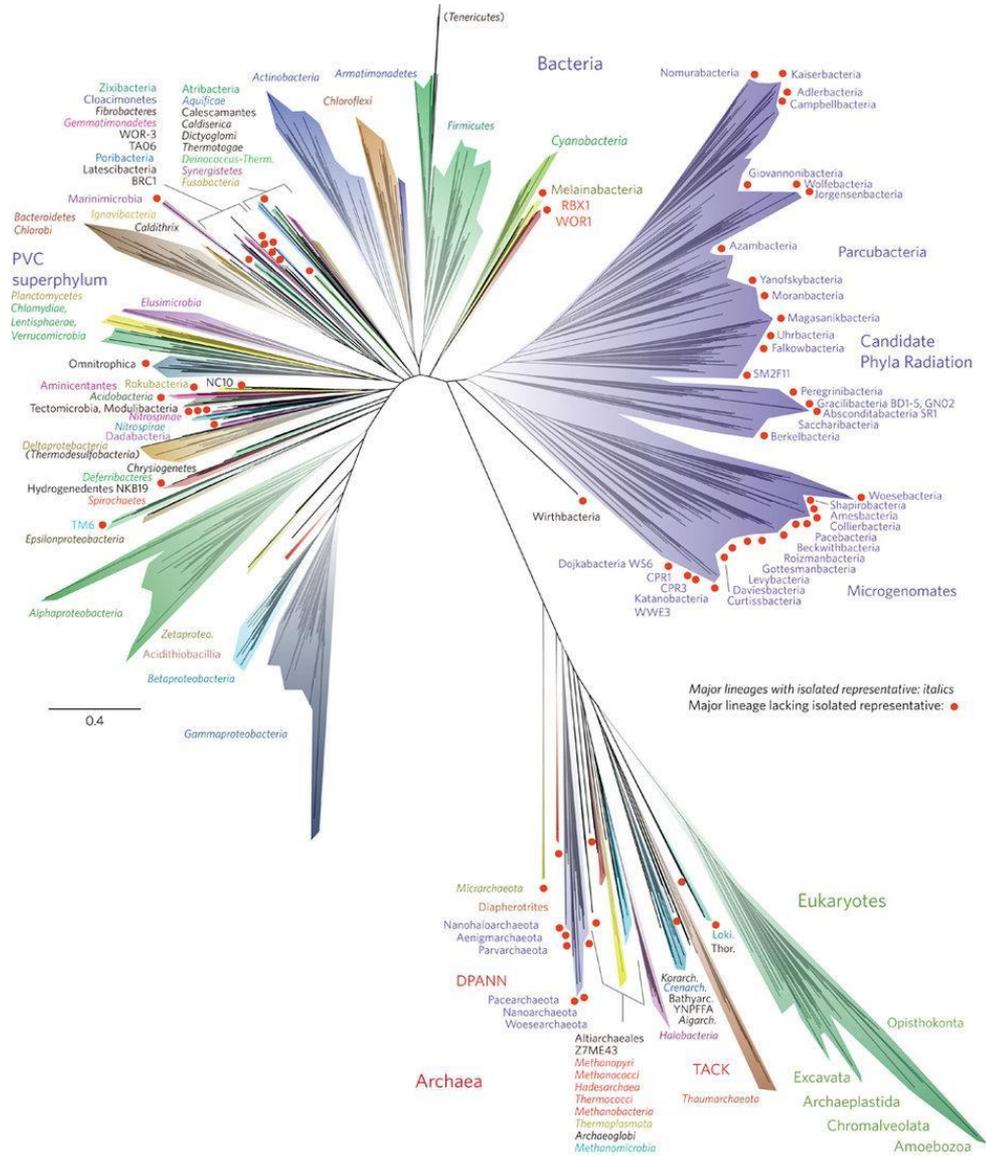
Hug et al., 2016.

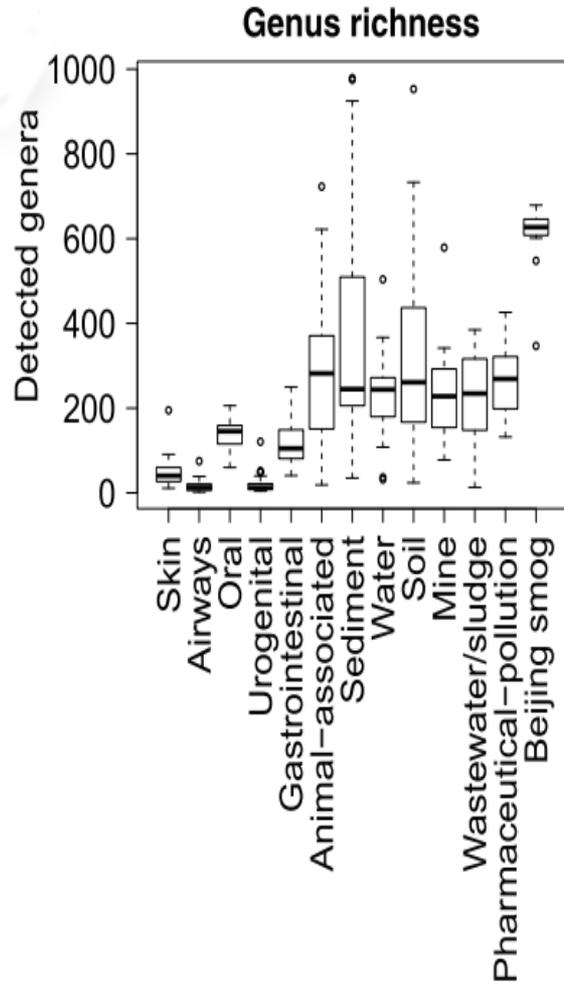


- Skin
- Airways
- Oral
- Urogenital
- Gastrointestinal
- Animal-associated
- Sediment
- Water
- Soil
- Mine
- Wastewater/sludge
- Pharmaceutical pollution
- Beijing smog

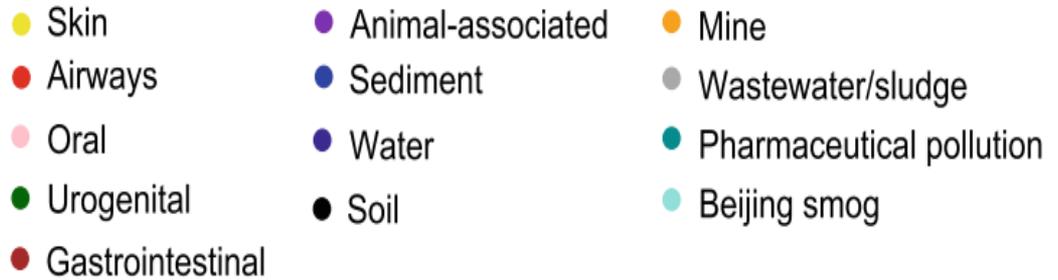
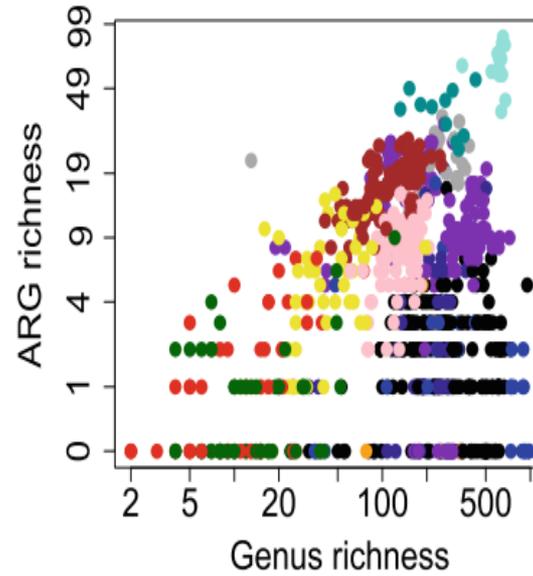


Pal et al., 2016.

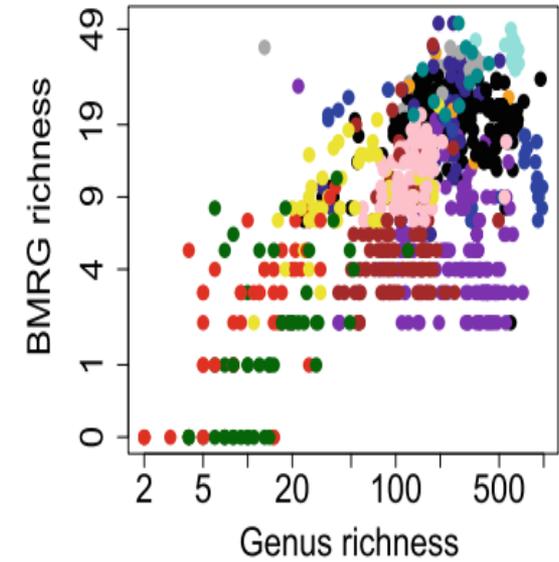




b



c



→ Soil and human microbiome as a diverse reservoir of ARG (Antibiotic Resistance Genes)

→ Soil genes associated with ARG

The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens

Kevin J. Forsberg,^{1*} Alejandro Reyes,^{1*} Bin Wang,^{1,2} Elizabeth M. Selleck,³ Morten O. A. Sommer,^{4,5†} Gautam Dantas^{1,2†}

Table 1. Nonredundant antibiotic resistance genes with 100% identity to known human pathogens.

Gene name	GenBank ID	Number of selections*	Antibiotic class	Annotation [mechanism]	Pathogens hit (GI number)
AB95_PI_68.1	JX009363	4	β-lactam	blaP1 [enzymatic degradation]	<i>A. baumannii</i> (94960156), <i>K. pneumoniae</i> (114147191), <i>P. aeruginosa</i> (117321883), <i>S. typhimurium</i> (12719011), <i>P. mirabilis</i> (157674381)†
AB95_CH_13.1	JX009364	1	Amphenicol	Chloramphenicol efflux [efflux]	<i>A. baumannii</i> (169147133), <i>P. aeruginosa</i> (260677483)
AB95_TE_2.2	JX009366	3	Tetracycline	tetA(G) [efflux]	<i>A. baumannii</i> (169147133), <i>S. typhimurium</i> (12719011)
AB95_TE_1.1	JX009365	3	Tetracycline	tetA [efflux]	<i>A. baumannii</i> (169147133), <i>E. coli</i> (312949035), <i>K. pneumoniae</i> (290792160), <i>S. typhimurium</i> (37962716)†
AB95_GE_3.3	JX009367 JX009373	2	Aminoglycoside	aadB [covalent modification]	<i>E. cloacae</i> (71361871), <i>K. pneumoniae</i> (206731403), <i>P. aeruginosa</i> (37955767), <i>S. typhimurium</i> (17383994)†
AB95_GE_3.1	JX009368 JX009374	2	Sulfonamide	sul1 [target modification]	<i>C. diptheriae</i> (323714042), <i>E. cloacae</i> (71361871), <i>K. pneumoniae</i> (206731403), <i>P. aeruginosa</i> (37955767), <i>S. typhimurium</i> (17383994), <i>Yersinia pestis</i> (165913934)†
AB95_CH_21.1	JX009369	1	Aminoglycoside	aacA4 [covalent modification]	<i>A. baumannii</i> (164449567), <i>K. pneumoniae</i> (238865601), <i>P. aeruginosa</i> (219872982), <i>S. typhi</i> (34014739)†

*Number of selections in which the entirety of a given gene was captured. †More pathogens exist for which 100% nucleotide identity was observed than listed

ARTICLE

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Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota

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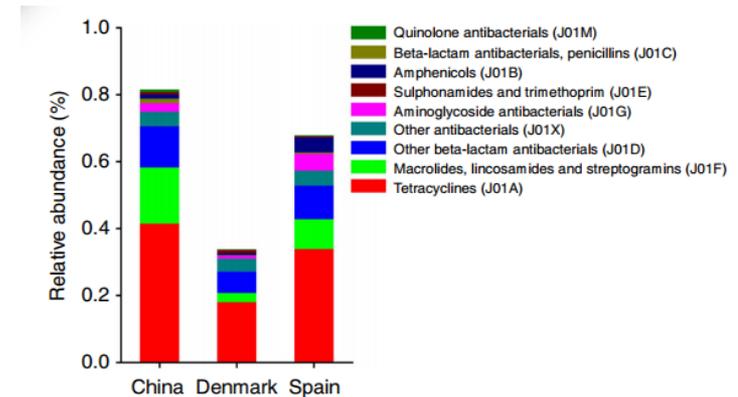


Figure 5 | The relative abundance of antibiotic resistance gene types assigned to each major antibiotic class in the different populations.

Resistance gene types were mapped to antibiotics according to the ARDB, and the classification of antibiotics was based on WHO ATC code J01. The average abundance for each gene type among individuals was used for mapping (excluding outliers). Resistances determined by more than one gene are not included, for example, by the vancomycin resistance operon. China: $n = 37$; Denmark: $n = 80$; Spain: $n = 36$.

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†More pathogens exist for which 100%

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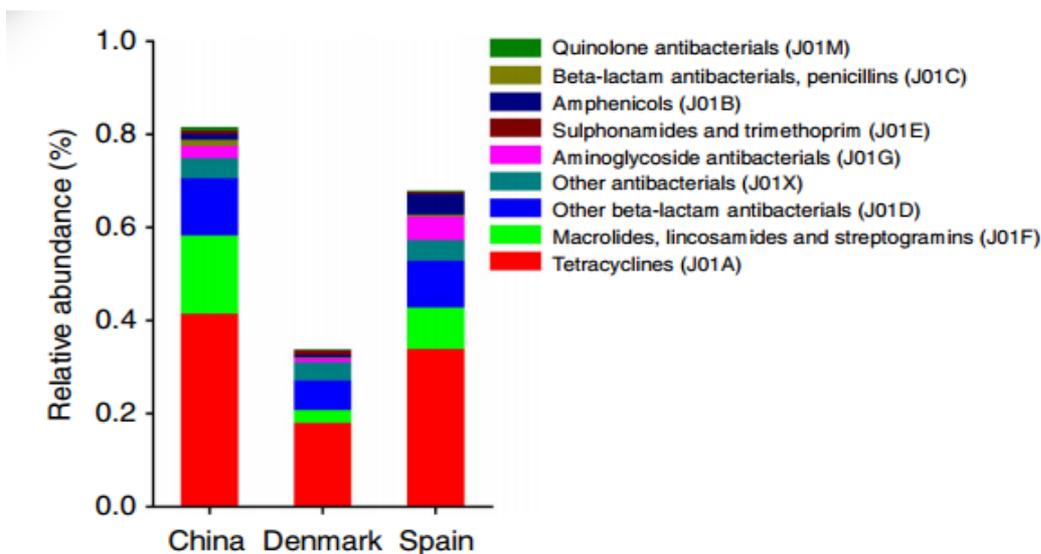
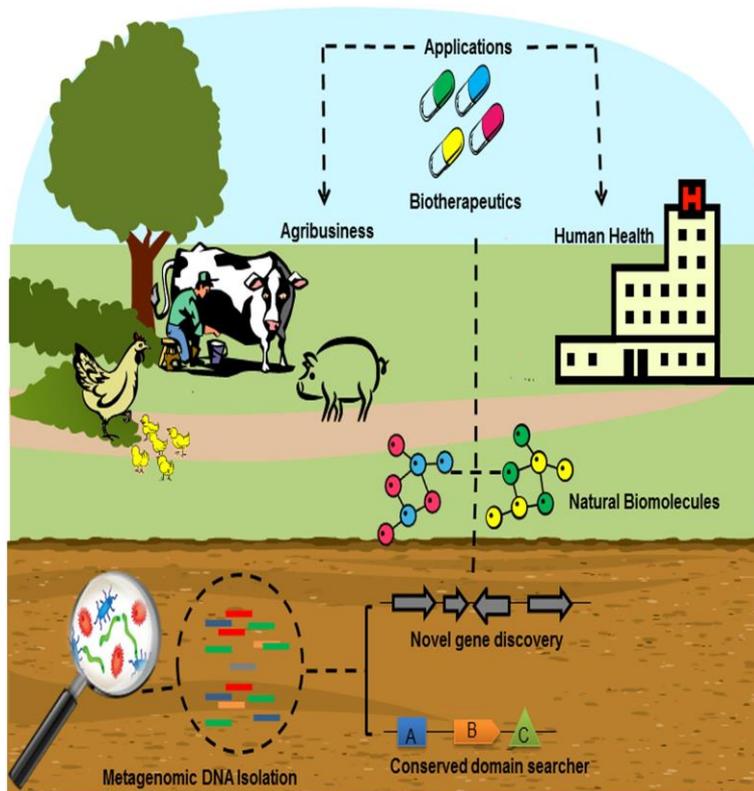


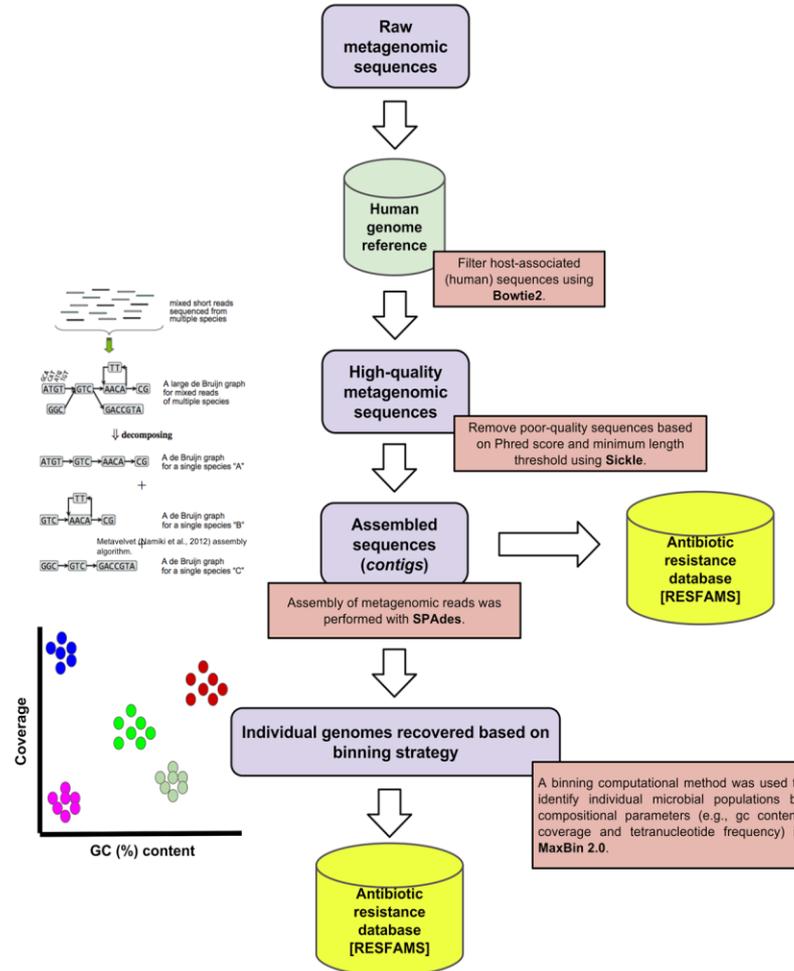
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- Bioprospecting in the *multi'omics* age:
- 1) High-throughput sequencing technologies
 - 2) Supercomputers and Bioinformatics tools to mining big data



→ **New perspectives:** Development of new computational approaches to discovery Antibiotic Resistance Genes (ARG).



Raw metagenomic sequences



Human genome reference

Filter host-associated (human) sequences using **Bowtie2**.



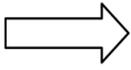
High-quality metagenomic sequences

Remove poor-quality sequences based on Phred score and minimum length threshold using **Sickle**.



Assembled sequences (*contigs*)

Assembly of metagenomic reads was performed with **SPAdes**.



Antibiotic resistance database [RESFAMS]

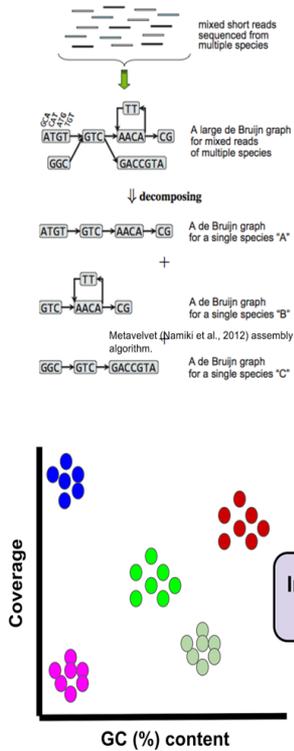


Individual genomes recovered based on binning strategy

A binning computational method was used to identify individual microbial populations by compositional parameters (e.g., gc content, coverage and tetranucleotide frequency) in **MaxBin 2.0**.



Antibiotic resistance database [RESFAMS]

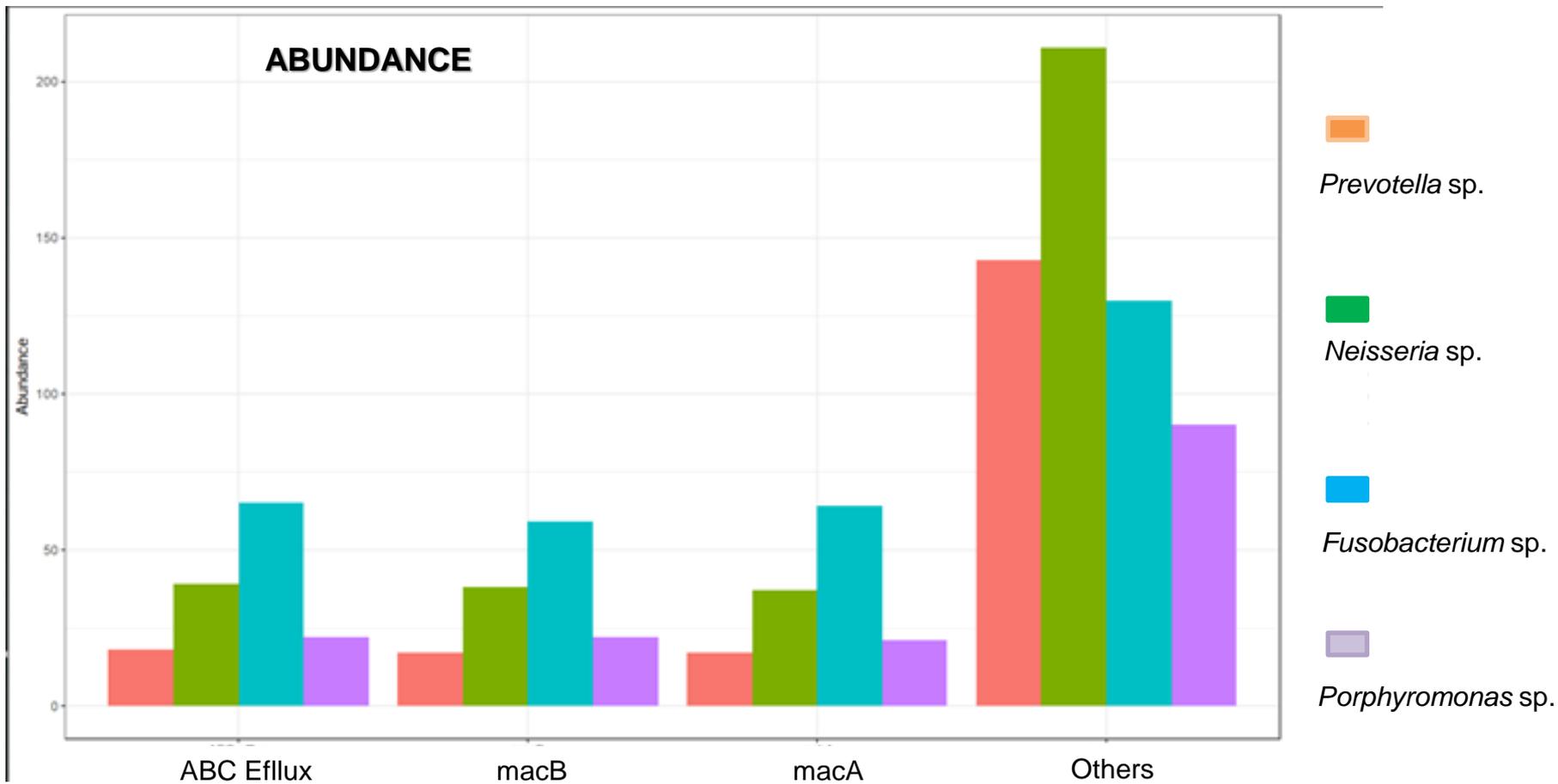


STUDY CASE I

The Oral Resistome

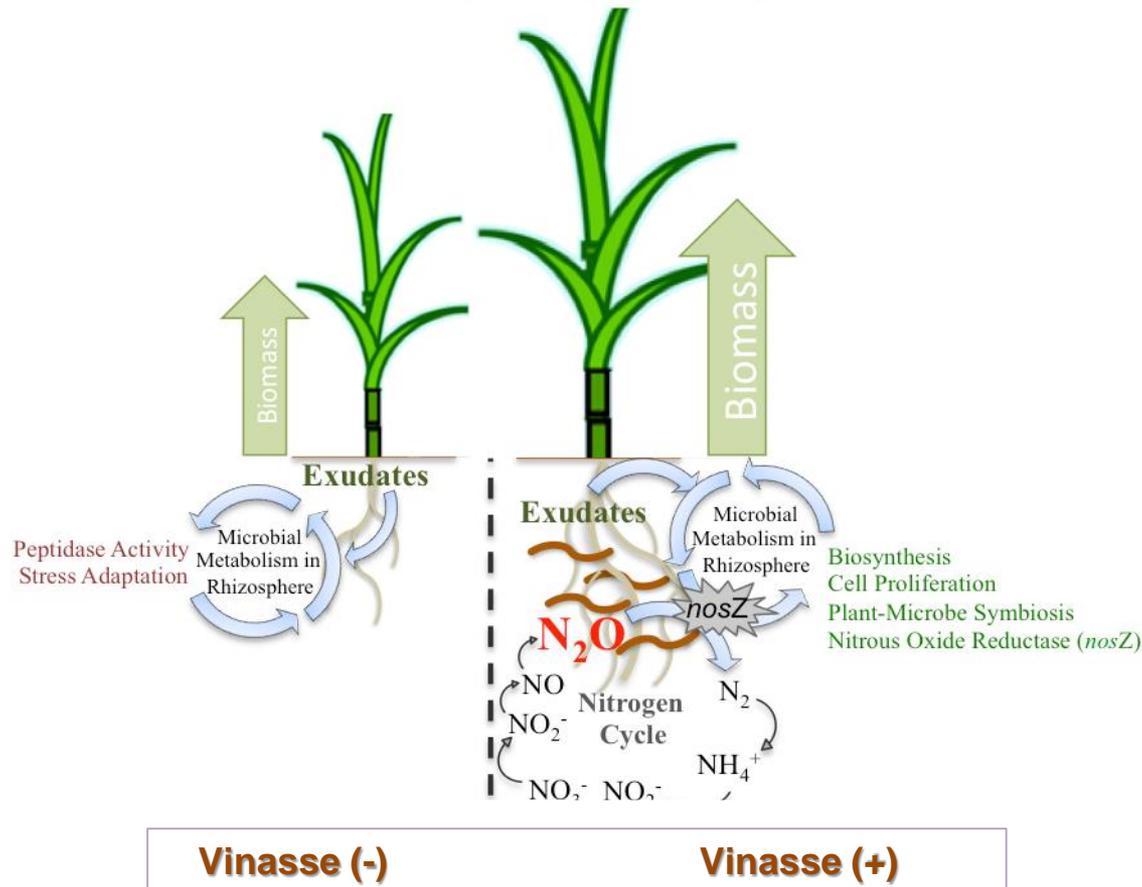
Genome name	BIN04	BIN06	BIN29	BIN35
Estimated Genome Size (Mb)	2.00	2.11	2.77	2.14
Number of contigs	154	64	1344	585
Best hit (16S rRNA) Identity/Coverage	<i>Prevotella</i> sp.	<i>Neisseria</i> sp.	<i>Fusobacterium</i> sp.	<i>Porphyromonas</i> sp.
Coverage/Identity (%)	(97/99)	(99/98)	(94/100)	(99/92)
Estimated Completeness (%)	62.11	99.92	71.87	94.13
Estimated Contamination (%)	2.01	1.20	5.62	5.50
G+C content (%)	40.0	49.3	29.8	56.3
Maximum scaffold length (bp)	183,899	376,899	18,699	22,799
N50 contig length	41,742	148,495	2,214	4,295
CDS number	1,661	2,106	2,593	1,762

GENOMES



STUDY CASE II

Vinasse fertirrigation alters soil resistome dynamics: an analysis based on metagenomic profiles



Vinasse fertirrigation alters soil resistome dynamics: an analysis based on metagenomic profiles

Soil resistome dynamics revealed by the response of antibiotic resistance genes (ARGs) to vinasse amendment

The error bar plots indicate the ARGs that changed abundance significantly (Welch's test, p -value < 0.05)

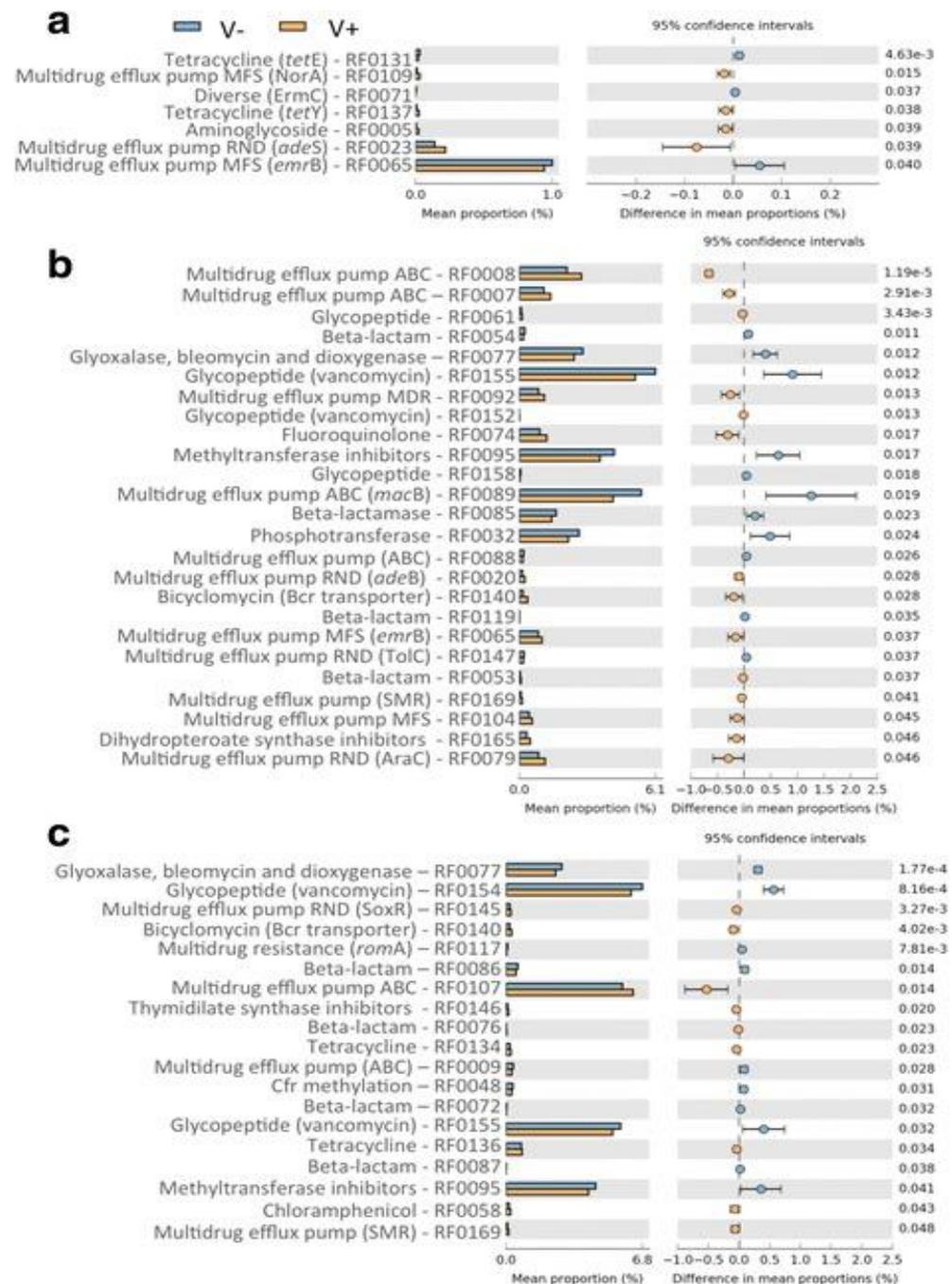


Vinasse (-)

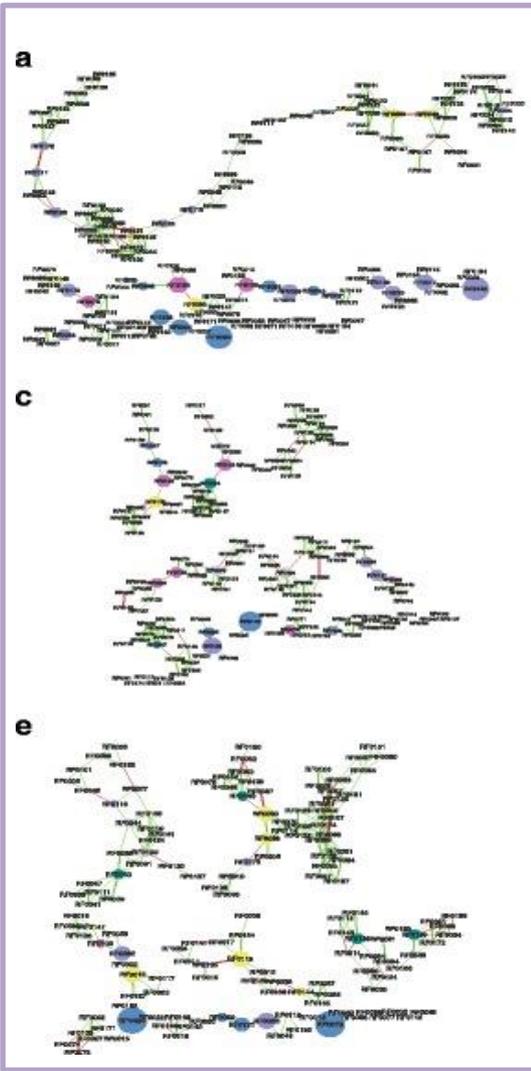


Vinasse (+)

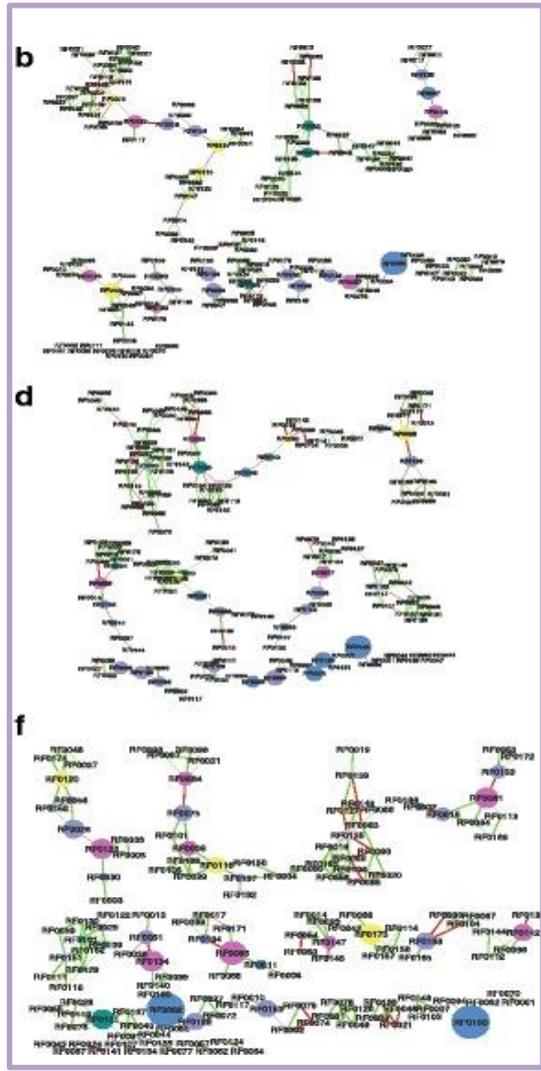
Braga et al. (2017)



VINASSE (-)



VINASSE (+)



Ecological interactions within the communities of antibiotic resistance genes (ARGs) predicted by network models. The models were constructed using the ARGs profiles from conditions without vinasse (V-) and with vinasse (V+) detected at the 7th day after planting **a**) (V-) and **b**) (V+), at the 157th dap **c**) (V-) and **d**) (V+) and at the 217th dap **e**) (V-) and **f**) (V+). The nodes size and color distinguishes their values of betweenness centrality (BC), the higher the BC the bigger the node, and their degree (number of connections), respectively