Organic fertilizers and irrigation water - a reservoir of antibiotic residues, resistance genes and mobile genetic elements affecting the resistome of plants?

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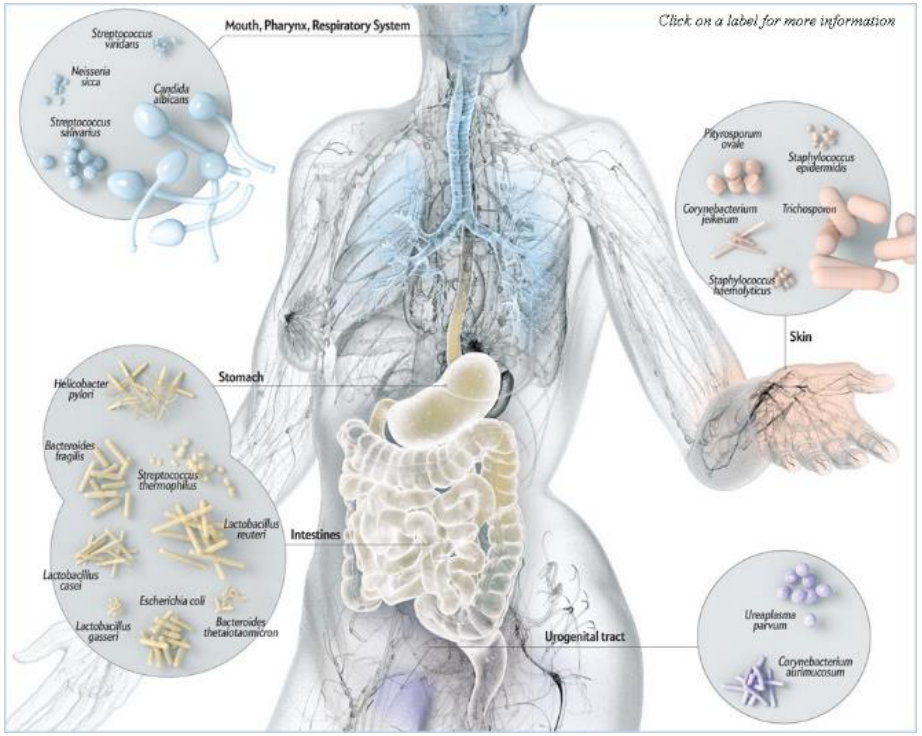
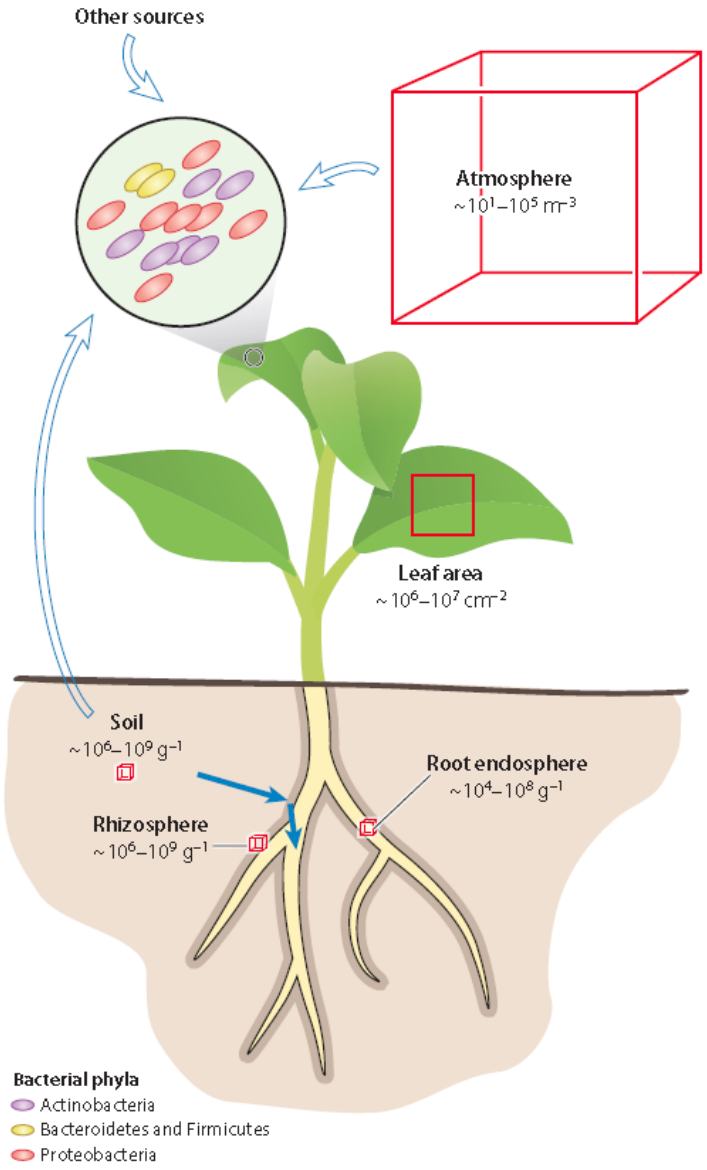
Antibiotic resistance is an ecological and environmental phenomenon





The natural or intrinsic resistome

comprises a set of elements that directly or indirectly contributes to antibiotic resistance: Lack of target, inactivation, low uptake and efflux (Olivares et al., 2013; Perry & Wright, 2013)

Acquired antibiotic resistance genes (ARGs)

Antibiotics, metal and biocide compounds used by humans cause community shifts, select resistance and horizontally acquired resistances

Humans create hotspots for bacterial evolution

- Waste water, sewage sludge, manure, digestates release bacteria carrying resistance genes often associated to mobile genetic elements and micro-pollutants such as disinfectants, pharmaceuticals (antibiotics) and heavy metals.

- This creates a hotspot for complex interactions between bacteria and their DNA elements in an environment containing sub-inhibitory concentrations of diverse selective agents.

Courtesy M. Gillings (modified)

Monitoring the transmission of antibiotic resistant bacteria, their antibiotic resistance genes & mobile genetic elements

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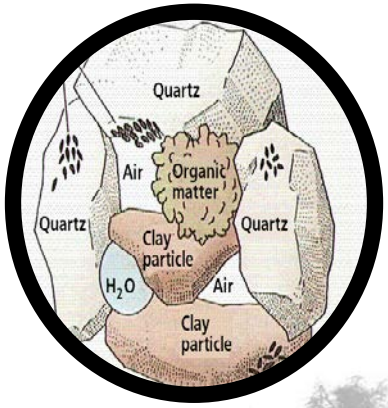
Organic fertilizer → **Soil** → **Crops** → **Fresh produce** → **Human gut microbiome**



Agricultural management practice:
Manure
Digestates
Biosolids
Irrigation water
Hydroponic production

Tools for studying antibiotic resistance genes and plasmids in organic fertilizers, irrigation water in plant and soil associated bacteria

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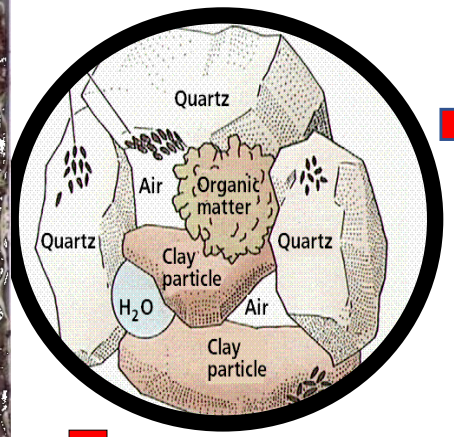
Cultivation-independent

Cultivation-dependent methods



DNA extraction

Metagenome

PCR-Southern blot
quantitative real-time PCR
HT-qPCR (Y-G Zhu)
16S amplicon sequencing

Plating with or without prior enrichment

Exogenous capturing of conjugative or mobilizable plasmids
→ Molecular characterization

Organic fertilizers - a reservoir of antibiotic residues, resistance genes and mobile genetic elements?

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Plasmids, integrons and antibiotic residues in piggery manure from different farms in Northern Germany

Farm	IncN	IncP-1	IncQ	IncW	LowGC	*intI1*	*intI2*	Detected antibiotics [mg/kg DW]
F1	-	+++	+++	-	-	+++	+++	Tc 300.00
F2	-	+++	+++	+++	-	+++	+++	Doxy 20.30
F3	-	+++	+++	+++	-	+++	+++	Tc 52.90
F4	-	+++	+++	-	-	+++	+++	Tc 161.00
F5	-	++	+	-	-	+++	+++	Tc 263.00
F6	-	+++	+++	-	-	+++	+++	Tc 287.00, SDZ 0.72, Ac-SDZ 11.50
F7	-	+++	+	-	-	+++	+++	Tc 5.50, CTc 26.60
F8	-	+++	+++	+++	-	+++	+++	-
B1	+	+++	+++	+++	+	+++	+++	Doxy 50.00
B2 Cellar	-	+++	+++	+++	-	+++	+++	Tc 4.60, Doxy 19.00
Silo	+	+++	+++	+++	-	+++	+++	Tc 9.40
B3 piglets	-	+++	+++	+++	-	+++	+++	Oxy 211.00
fatten.	+	+++	+++	+++	-	+++	+++	CTc 15.80, Oxy 14.90
B4 Silo a	+	+++	+++	-	-	+++	+++	CTc 26.40, Doxy 27.70
Silo b	+	+++	+++	+++	-	+++	+++	Doxy 101.00
B5	++	+++	+++	+++	-	+++	+++	Tc 1.50, Enr 1.30, Tia 1.40
B6	+	+++	+++	+++	-	+++	+++	Doxy 52.60, SDM 23.00

→IncN and lowGC plasmids only detected in manures from pig breeding

Organic fertilizers - a reservoir of antibiotic residues, resistance genes and mobile genetic elements?

Detection of antibiotic and qac resistance genes in piggery manure from different farms in Northern Germany

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Farm	*sul1*	*sul2*	*sul3*	*tet*(A)	*tet*(M)	*tet*(X)	*qacE*	*qacE*Δ*1*
F1	+++	+++	-	++	++	++	-	+++
F2	+++	+++	-	++	++	++	-	+++
F3	+++	+++	-	++	++	++	-	+++
F4	+++	+++	-	++	++	++	-	+++
F5	+++	+++	-	++	+	++	-	+++
F6	+++	+++	-	++	+	++	-	+++
F7	+++	+++	-	++	+	++	-	+++
F8	+++	+++	-	++	+	++	-	+++
B1	+++	+++	-	++	+	++	-	+++
B2								
Cellar	+++	+++	-	++	+	++	(+)	+++
Silo	+++	+++	-	++	(+)	++	-	+++
B3								
Piglets	+++	+++	-	++	+	++	-	+++
Fatten.	+++	+++	-	++	+	++	-	+++
B4 Silo a	+++	+++	-	++	+	++	-	++
Silo b	+++	+++	-	++	+	++	-	+++
B5	+++	+++	-	++	+	++	-	+++
B6	+++	+++	-	++	++	++	-	+++

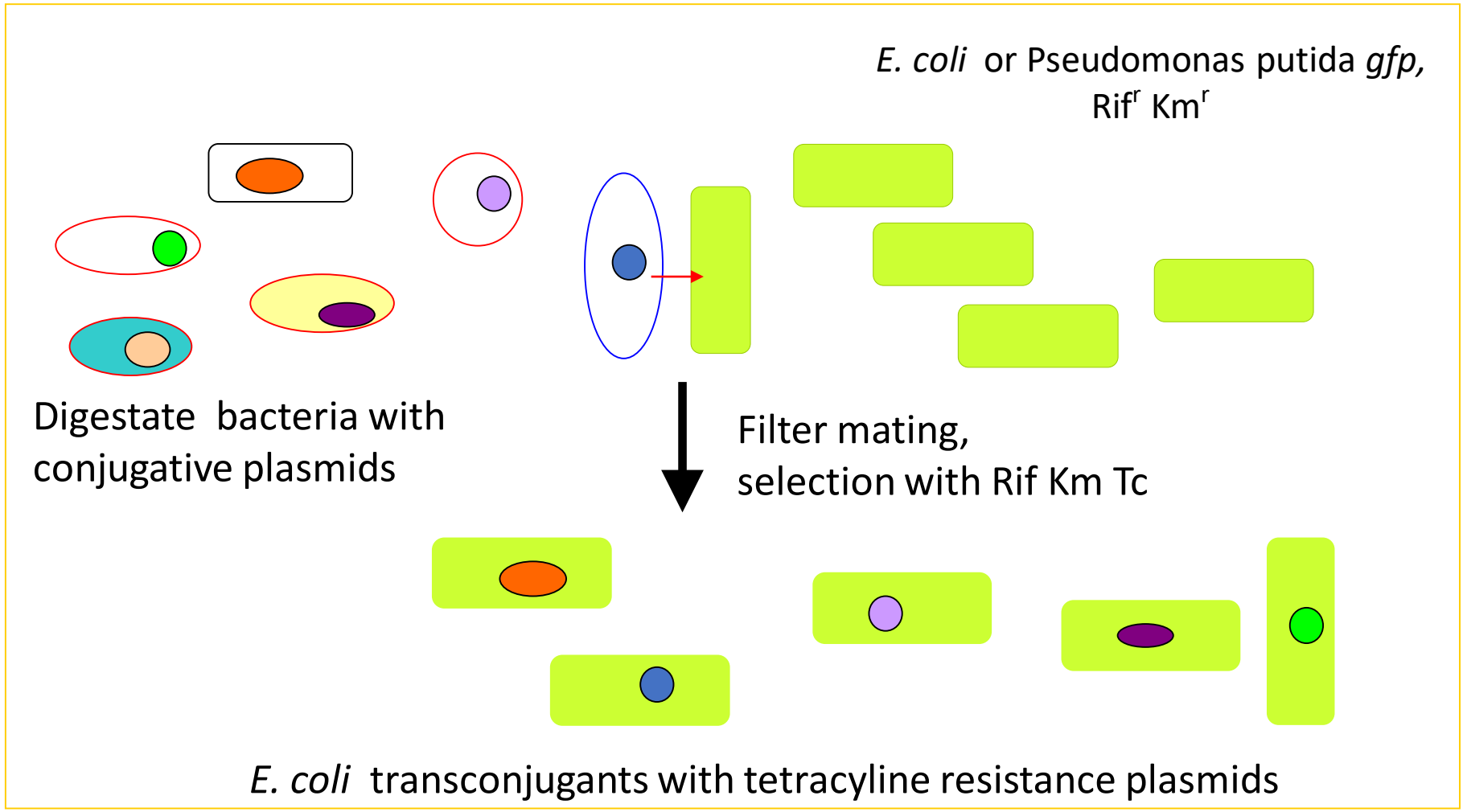
Detection of plasmids, integrons and antibiotic residues in digestates from biogas plants in Northern Germany

Farm	Source	IncN	IncP-1	IncQ	IncW	LowGC	*intI1*	*intI2*	*Detected antibiotics [mg/kg DW]*
BGP1	Digestate	-	+	+++	++	-	++	++	Tc 1.60, Doxy 1.30
BGP2	Digestate	-	+	+++	++	(+)	++	++	Doxy 7.40
BGP3	Digestate	-	+	+++	+	-	++	++	Doxy 2.10
BGP4	Digestate	-	+	+++	+	-	++	++	Tc 2.10, Doxy 10.10, Enr 0.20
BGP5	Digestate	-	(+)	+++	++	(+)	++	++	Doxy 3.20
BGP6	Digestate	-	(+)	+++	++	+	++	++	Tc 6.40, Doxy 2.20
BGP7	Digestate	-	-*	+++	++	-	++	++	Tc 0.41
BGP8	Digestate	-	(+)	+++	++	-	++	++	-

*: IncP-1$_{\varepsilon}$ plasmids were captured from this digestate via exogenous plasmid isolation

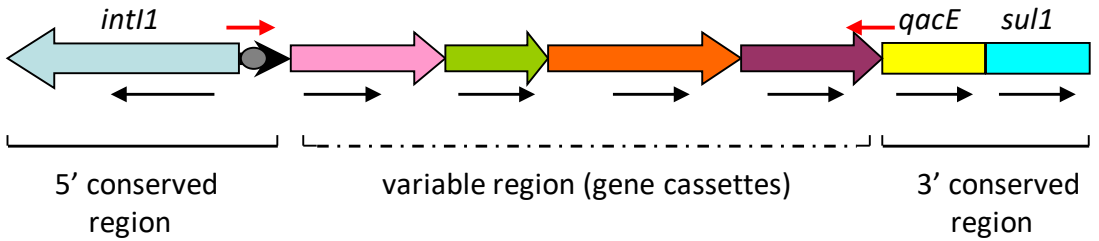
➡ Except for IncN plasmids (no detection) and LowGC plasmids (detected in 3 out of 8 digestates), all mobile genetic elements monitored were detected in all samples.

Transferable resistome of digestates exogenously captured



All plasmids exogenously captured from digestates of different biogas fermentation plants belonged to the IncP-1e group

# TF	BGP	Detected markers	Gene cassette size [bp]	Resistance	Moderate resistance	# rp
Ps26	2	*intI1*, *qacEΔ1*, *sul1*, *tet*(A), *aadA1*	1000	SDZ, TET*	DOX	3
Ps28	2	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	1600	SDZ, TET*, TMP	DOX, SM	1
Ps29	2	*intI1*, *qacEΔ1*, *sul1*, *tet*(A), *aadA1*	2300 + 1700	SDZ, CM, SM, TET*	DOX	2
Ps32	2	*intI1*, *qacEΔ1*, *sul1*, *tet*(A), *aadA1*	1000	SDZ, SM, TET*	DOX	4
Ps101	5	*intI1*, *qacEΔ1*, *sul1*, *tet*(A), *aadA1*	1700	SDZ, SM, TET*	DOX, CM	5
Ps110	5	*intI1*, *qacEΔ1*, *sul1*, *tet*(A), *aadA1*	1000	SDZ, TET*	DOX, SM	6
Ps151	6	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	1500	SDZ, TET*, TMP	DOX, SM	1
Ps152	6	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	2200	SDZ, TET*, TMP	DOX, SM	1
Ps154	6	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	2200	SDZ, TET*, TMP	DOX, SM	1
Ps156	6	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	2200	SDZ, TET*, TMP	DOX, SM	1
Ps128	7	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	2200	SDZ, TET*, TMP	DOX, SM	1
Ps134	7	*intI1*, *qacEΔ1*, *sul1*, *tet*(A), *aadA1*	2300 + 1700	SDZ, CM, SM, TET*	DOX	2
E9	1	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	2200	SDZ, TET*, TMP	DOX, SM	1
E10	1	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	2200	SDZ, TET*, TMP	DOX, SM	1
E12	1	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	2200	SDZ, TET*, TMP	DOX, SM	1
E17	1	*intI1*, *qacEΔ1*, *sul1*, *tet*(A)	2200	SDZ, TET*, TMP	DOX, SM	1



Class 1 Integrons

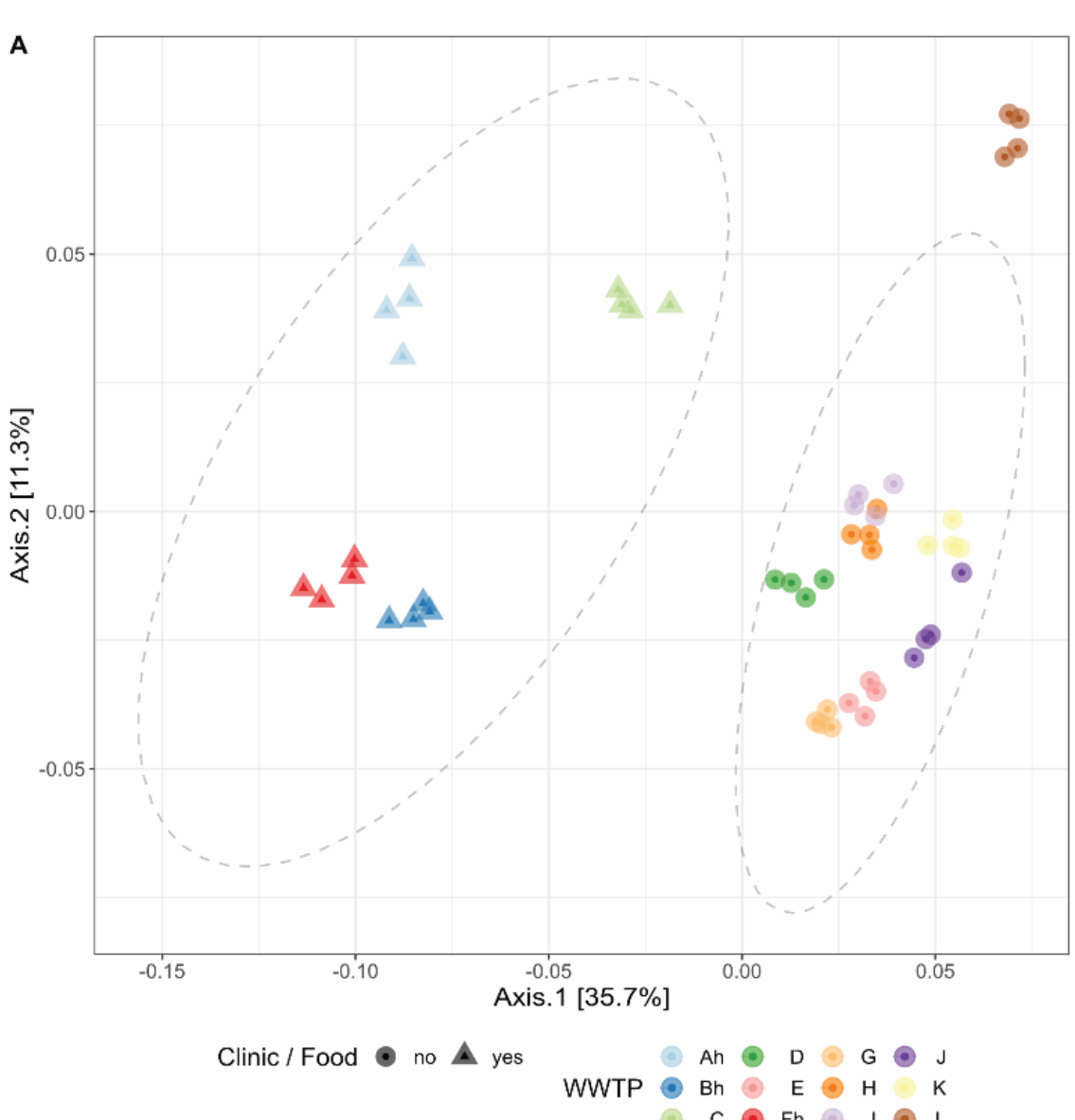
Wolters et al., 2015,
Fontiers in Microbiology

Biosolids for safe land application: does wastewater treatment plant size matter when considering antibiotics, pollutants, microbiome, MGE and associated resistance genes?

Wolters et al., Environ Microbiol 2022

WWTP	IEs*	Size	WWTP Treatment	State of biosolids	Hospital in catchment area	Biosolid disposal	Other features of catchment area
A^{L}	>100,000	Large	Anaerobic digestion	Dewatered	Yes	Land use	Hospital
B^{M}	<50,000	Medium	Anaerobic digestion	Liquid	Yes	Land use	Hospital, food industry
C^{L}	<100,000	Large	Aerobic stabilization	Liquid	No	Incineration	Food industry
D^{S}	< 10,000	Small	Aerobic stabilization	Liquid	No	Land use	
E^{M}	< 50,000	Medium	Aerobic stabilization	Liquid	No	Land use	
F^{M}	< 50,000	Medium	Anaerobic digestion	Dewatered	Yes	Land use	Hospital
G^{S}	< 10,000	Small	Aerobic stabilization	Liquid, non-stabilized**	No	Land use**	
H^{M}	< 50,000	Medium	Aerobic stabilization	Liquid	No	Incineration	
I^{S}	< 10,000	Small	Aerobic stabilization	Liquid	No	Land use	
J^{M}	< 50,000	Medium	Aerobic stabilization	Liquid	No	Land use	
K^{M}	< 50,000	Medium	Aerobic stabilization	Liquid	No	Land use	
L^{S}	< 10,000	Small	Aerobic stabilization	Liquid	No	Land use	Amino-acid production factory

Dissimilarity and composition of the WWTP biosolid samples' microbial communities

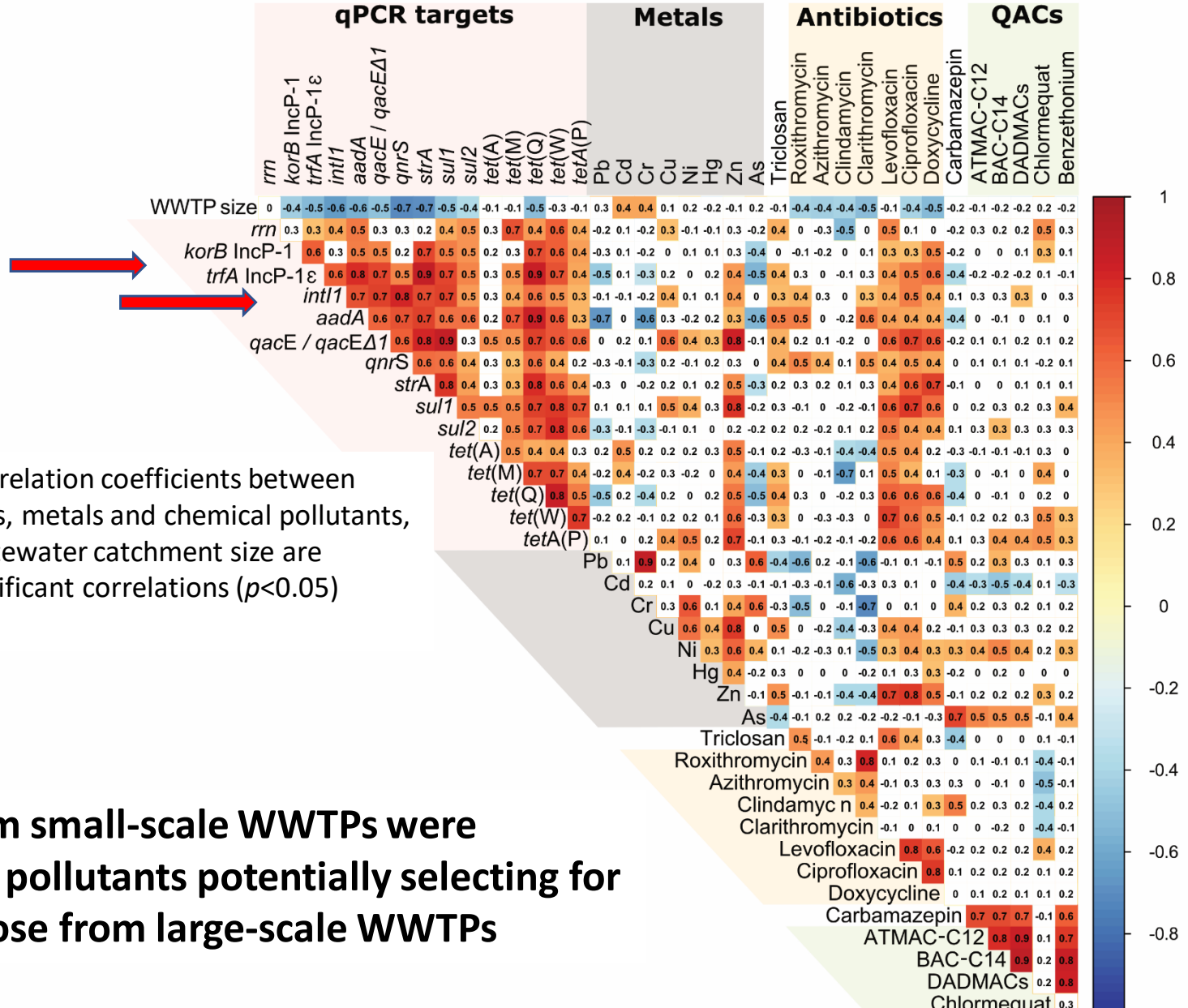


PCoA based on weighted UniFrac distance using between samples at the **ASV level**. Ellipses show the 95% confidence intervals delineating the two groups of samples from WWTPs with a hospital or food industry in their catchment area (triangles) or without (circles).

The clustering in the two groups is supported by permutational ANOVA (*p*-value < 0.001; R^2 = 23.6%)

Sludge processing and the presence of a hospital or a food industry in the catchment area had a strong influence on the microbial community composition

Correlogram displaying all correlations between measured environmental variables in liquid biosolid samples



Spearman's rank correlation coefficients between measured antibiotics, metals and chemical pollutants, RGs, MGEs and wastewater catchment size are shown and only significant correlations (p<0.05) are coloured.

Biosolids originating from small-scale WWTPs were no less contaminated by pollutants potentially selecting for ARGs and MGEs than those from large-scale WWTPs

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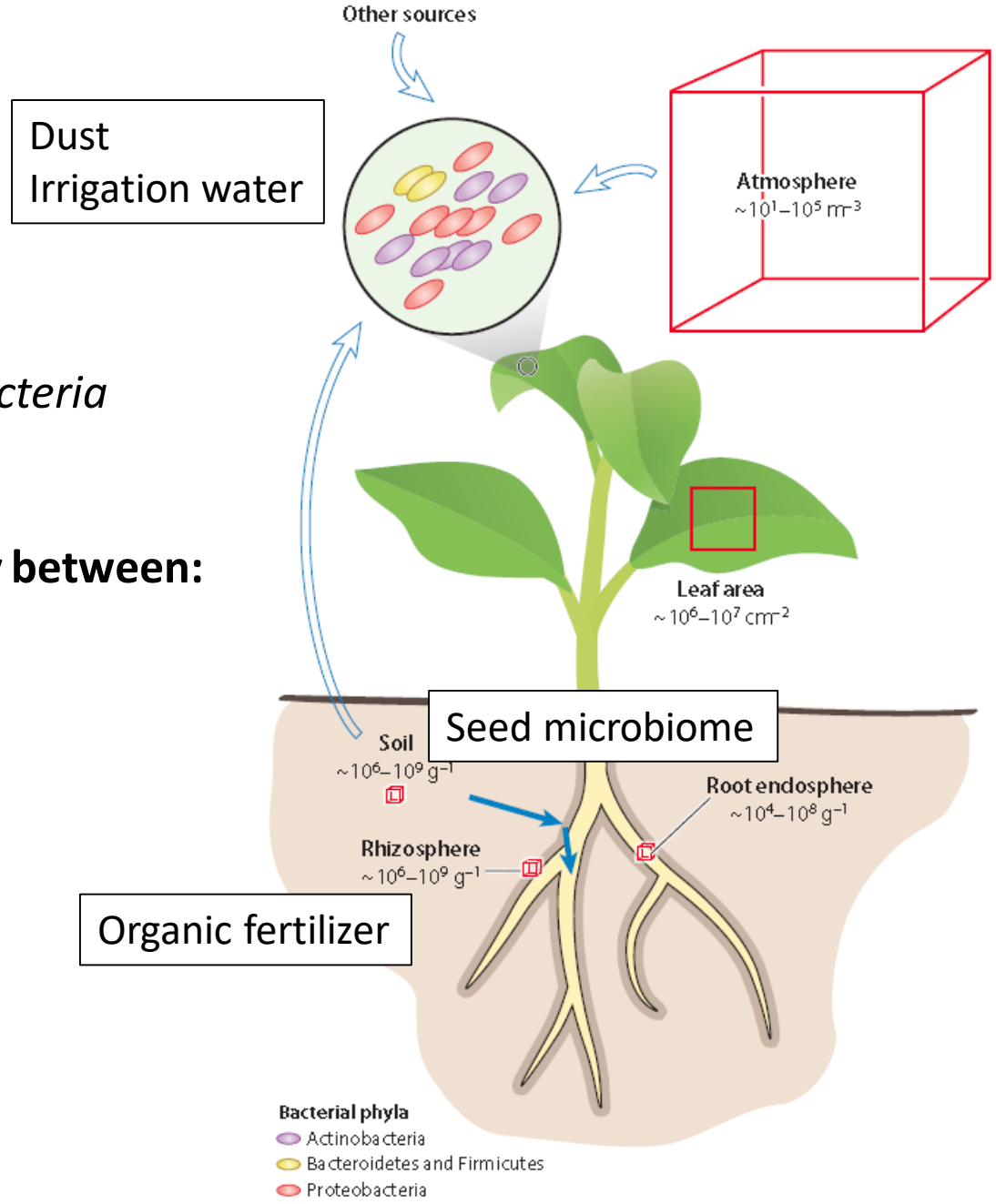
Plant microbiome

Diverse bacterial communities

Phyllosphere: 10^6-10^7 CFU/g

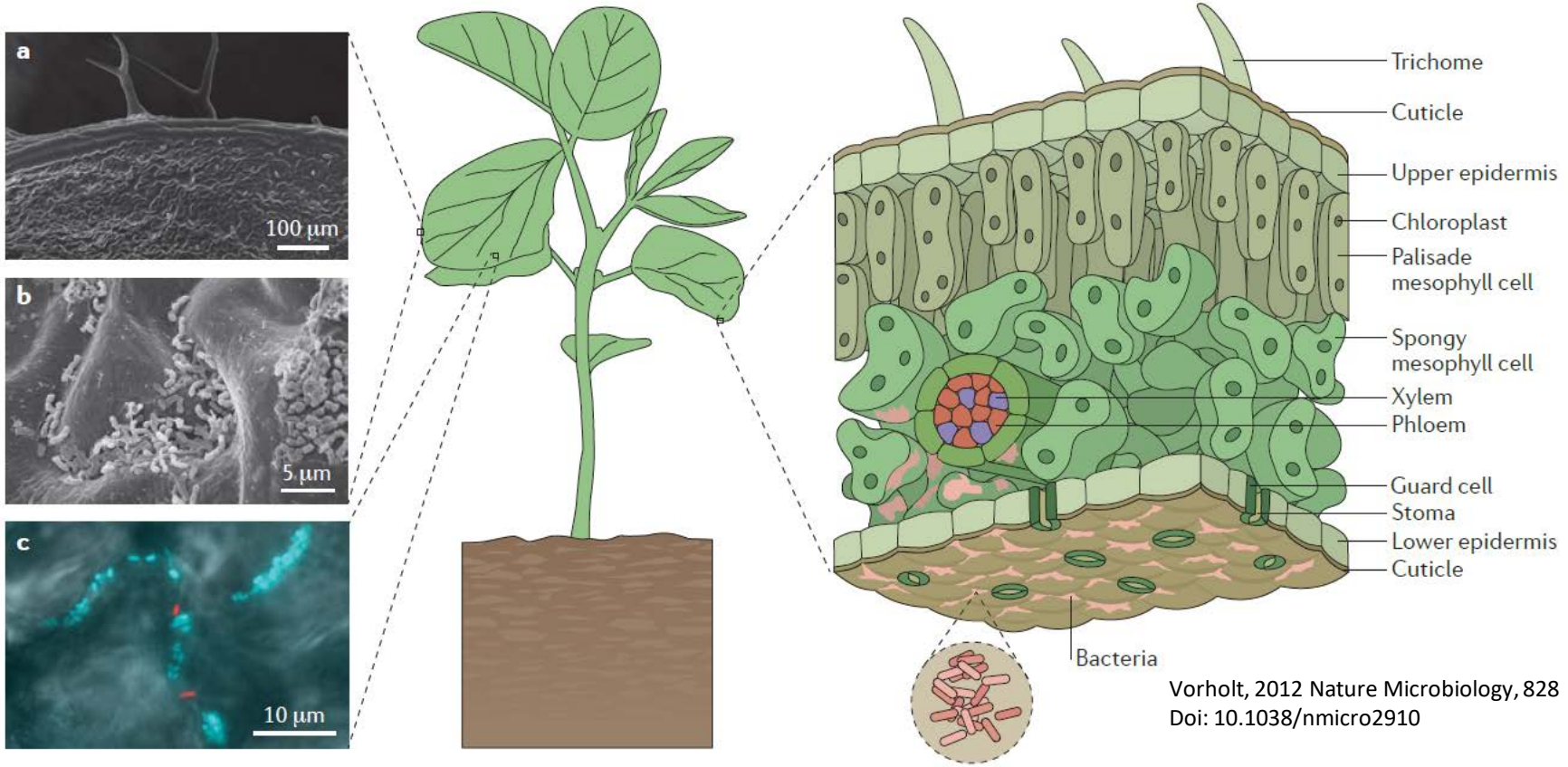
Dominant phyla: *Proteobacteria, Firmicutes, Actinobacteria Bacteroidetes*

Bacterial community composition differs significantly between:
Plant species
Cultivars
Plant developmental stage
External drivers
Complex microbial communities prevent invasion by human pathogens



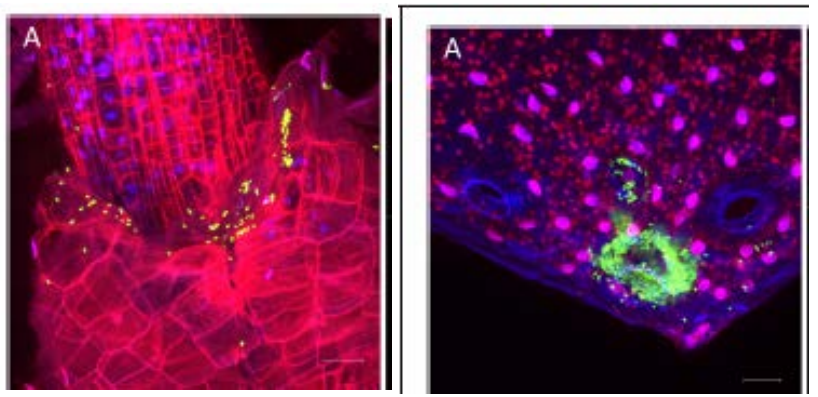
(Bulgarelli *et al.*, 2013; Leff and Fierer, 2013)

The phyllosphere



Vorholt, 2012 Nature Microbiology, 828
Doi: 10.1038/nmicro2910

a) SEM Arabidopsis leaf with trichomes
b) SEM of *Sphingomonas*
c) Epifluorescence of *Pantoea agglomerans* cfp and *Pseudomonas syringae* mCherry



- Patchy and heterogenous colonization patterns
- Locally high cell densities (aggregates, biofilms)
- Heterogenous distribution of nutrients

Jechalke et al., 2019. Front Microbiol doi: 10.3389/fmicb.2019.00967

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It is important to note that the causal agents of nosocomial infections such as

Acinetobacter
Burkholderia
Enterobacter
Klebsiella
Pseudomonas
Stenotrophomonas
Serratia

are typical members of the plant microbiome (Berg et al., 2005; Ryan et al., 2009).

Potential reasons for the success of plant associated bacteria in clinical settings:

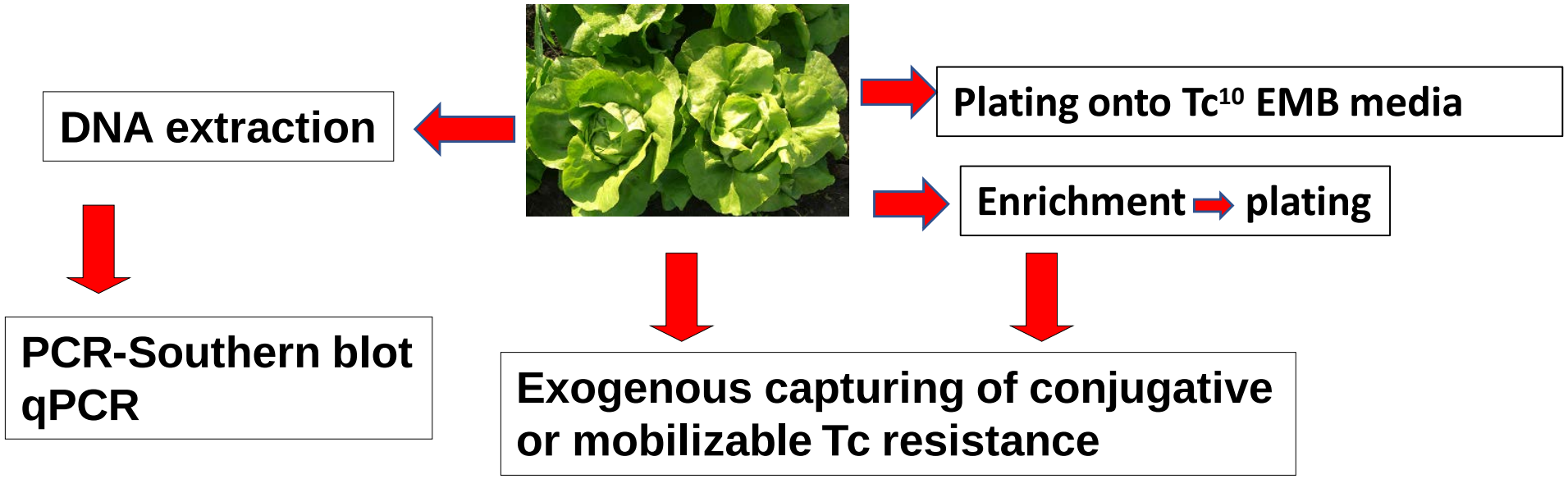
Many plant associated bacteria have the ability to form biofilms and display intrinsic resistances
They display multiple resistances often due to efficient efflux pumps

➡ enormous selective advantages under hospital condition.

The rare plant microbiome might also contain *E. coli* carrying transferable multiple resistance plasmids.

Sampling the transferable resistome of produce

Development of qPCR systems to detect IncF, IncI1, IncI2 as these plasmids have a host range restricted to *Enterobacteria* such as *E. coli*



Blau, K. et al.,. 2018. The transferable resistome of produce. mBio 9:e01300-18. https://doi.org/10.1128/mBio.01300-18

Characterization of representative tetracycline resistant *E. coli* isolated from cilantro by plating

E. coli isolates	ST	Inc groups[a]	*bla* genes	Resistance and integrase genes	Antibiotic resistance profile[b]
EK2.1	0	**FII**[l]	bla_{TEM}	*tet*(A), *qnrS*	AM, AMX, TE, CIP, OFX
EK2.5	0	**I1**[l]	bla_{TEM}	*tet*(A), *qnrS*	AM, AMX, TE, CIP
EK2.7	0	U[h]	bla_{TEM}	*intI1*, *tet*(A), *sul2*, *strA*, *qnrS*	AM, AMX, TE, S, TMP, SD, OFX
EK2.8	0	**I1**[l]	bla_{TEM}	*intI1*, *tet*(A), *sul2*, *strA*	AM, AMX, TE, S, TMP
EK2.11	0	I1[f]	bla_{TEM}	*tet*(A)	AM, AMX, TE
EK2.15	0	I1[f]	bla_{TEM}	*tet*(A), *sul2*	AM, AMX, TE
EK2.16	0	N[g]	bla_{TEM}	*tet*(A), sul2, sul3, *strA*	AM, AMX, TE, S
EK2.18	0	X1[h]	bla_{TEM}	*tet*(A), *sul2*, *sul3*, *strA*	AM, AMX, TE, S
EK3.34	0	**FII**[l]	bla_{TEM}	*intI1*, *tet*(A), *sul1*, *strA*	AM, AMX, TE, S, TMP, SD, NA, OFX
EK3.35	0	**FII**[l]	bla_{TEM}	*tet*(A), *sul1*	AM, AMX, TE, S, TMP, SD, NA

[a]: l: detected by RT-PCR and PBRT; f: detected by RT-PCR; g: detected by PCR; h: detected by PBRT.

[b]: AM: Ampcillin; AMX: Amoxicillin; TE: Tetracycline; S: Streptomycin; TMP: Trimethoprim; SD: Sulfadiazine; CIP: Ciprofloxacin; OFX: Ofloxacin; NA: Nalidixic acid.

Characterization of tetracycline resistant *E. coli* isolates from cilantro after enrichment

E. coli isolates	ST	Inc groups[a]	*bla* genes	Resistance and integrase genes	Antibiotic resistance profile[b]
EK2.2	0	U[h]	bla_{TEM}	*intI1, tet*(A), *sul2,strA, qnrS*	AM, AMX, TE, S, TMP, SD, CIP, OFX
EK2.19	0	FII[l]	bla_{TEM}	*intI1, tet*(A), *merRTΔP, sul2, aadA*	AM, AMX, TE, S, TMP, D, GM, KM
EK2.20	0	HI1[h]	bla_{TEM}	*intI1, tet*(A), *sul2*, *sul3*, *aadA, qnrS*	AM, AMX, TE, S, TMP, SD, GM, OFX, C
EK2.21	0	X1[h]	bla_{TEM}	*intI1, tet*(A)	AM, AMX, TE, OFX
EK2.22	0	X1[h]	bla_{TEM}	*intI1, tet*(A), *strA, qnrS*	AM, AMX, TE, S, TMP, SD, OFX
EK2.25	0	U[h], X1[h]	bla_{TEM}	*intI1, tet*(A), *sul2, strA, qnrS*	AM, AMX, TE, S, TMP, SD, CIP, OFX
EK2.26	0	U[h]	bla_{TEM}	*intI1, tet*(A), *sul1*, *aadA, qacEΔ1, strA*	AM, AMX, TE, S, TMP, SD, CIP, NA, OFX
EK2.29[k]	0	N[g]	bla_{TEM}, $bla_{CTX-M-1}$	*intI1, tet*(A), *sul1*, *strA, qnrS*	AM, AMX, TE, S, TMP, SD, CRO, CTX, OFX, CIP
EK2.30	0	U[h]	bla_{TEM}	*intI1, tet*(A), *sul1, sul3*, *aadA, qnrS*	AM, AMX, TE, TMP, SD, C
EK3.33	0	X1[h]	-	*intI1, sul1, strA*	TE, D
EK3.36	0	**FIB[l]**	bla_{TEM}	*intI1, tet*(A), *merRTΔP, sul1, aadA*	AM, AMX, TE, S, TMP, D, GM, KM
EK3.43[k]	0	N[g]	$bla_{CTX-M-1}$	*tet*(A), *sul1,qnrS*	AM, AMX, TE, S, D, GM, CTX, OFX
EK3.44[k]	7	N[g]	bla_{TEM}, $bla_{CTX-M-1}$	*intI1, tet*(A), *merRTΔP, sul1, strA, qnrS*	AM, AMX, TE, S, TMP, SD, CRO, CTX, C, NA, CIP, OFX
EK5.16	7	**FII[l]**, I1[f]	bla_{TEM}	*intI1, tet*(A), *aadA*, *qacEΔ1, qnrS*	AM, AMX, TE, S, TMP, D, OFX
EK5.19	7	X1[h]	bla_{TEM}	*intI1, tet*(A), *sul2, strA, qnrS*	AM, AMX, TE, S, TMP, SD, D, C
EK5.20	7	**FII[l]**, I1[f]	bla_{TEM}	*intI1, tet*(A), *aadA, qacEΔ1, qnrS*	AM, AMX, TE, S, TMP, OFX
EK5.25	7	**FII[l]**	bla_{TEM}	*intI1, tet*(A), *qacEΔ1, qnrS*	AM, AMX, TE, TMP, OFX
EK5.28	7	**FII[l]**, I1[f]	bla_{TEM}	*intI1, tet*(A), *aadA, qacEΔ1, qnrS*	AM, AMX, TE, S, TMP, CIP, OFX
EK5.30	7	**FII[l]**, FIB[l], I1[f]	bla_{TEM}	*intI1, tet*(A), *sul1, aadA, qacEΔ1, qnrS*	AM, AMX, TE, TMP, SD, D, CIP, NA, OFX
EK5.32	7	X1[h]	bla_{TEM}	*intI1, tet*(A), *sul1*, *sul2, strA, qnrS*	AM, AMX, TE, S, TMP, SD, OFX, C
EK5.40	7	X1[h]	bla_{TEM}	*intI1, tet*(A), *sul2, strA, qnrS*	AM, AMX, TE, S, TMP, SD, C
EK7.6	7	U[h]	bla_{TEM}	*intI1, tet*(A)	AM, AMX, TE, TMP, D
EK7.7	7	I1[f]	bla_{TEM}	*intI1, tet*(A), *qacEΔ1, aadA*	AM, AMX, TE, TMP
EK7.9	7	X1[f]	bla_{TEM}	*intI1, tet*(A), *merRTΔP, sul2, qnrS*	AM, AMX, TE, SD, CRO, D, CIP, C, GM, OFX
EK7.10	7	HI1[h]	bla_{TEM}	*intI1, tet*(A), *merRTΔP, sul2, qnrS*	AM, AMX, TE, SD, CRO, D, CIP, C, GM, OFX

Whole Genome Sequencing of 120 *E. coli isolates* from produce

Phylogroup B1 dominated

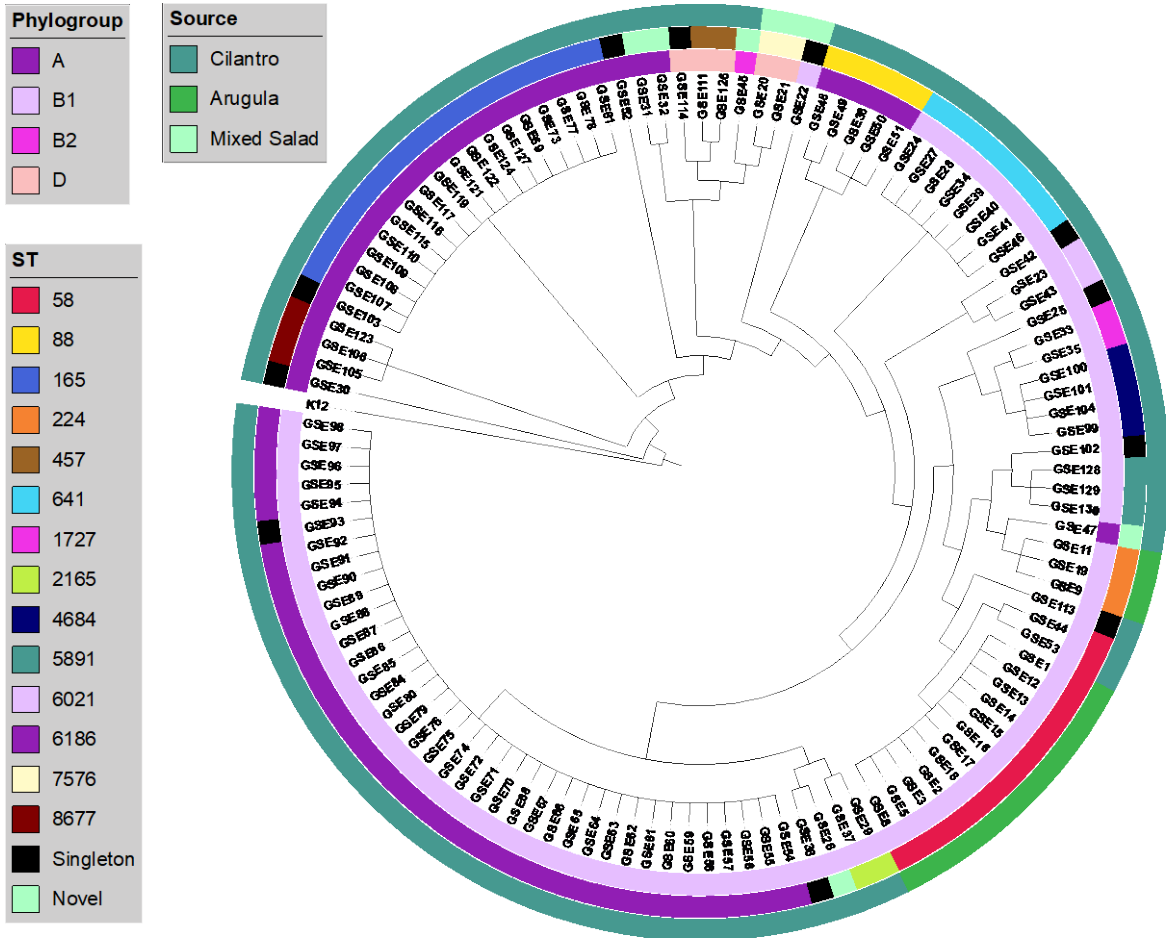
common STs:

6186, 165, 58, 641, 88

84 of the strains carried *intI1*

All ST6186 strains originating from cilantro carried *intI*1 with variable ARGs

ST58: 2 of 14 strains carried ESBL gene *bla*$_{CTX-M15}$

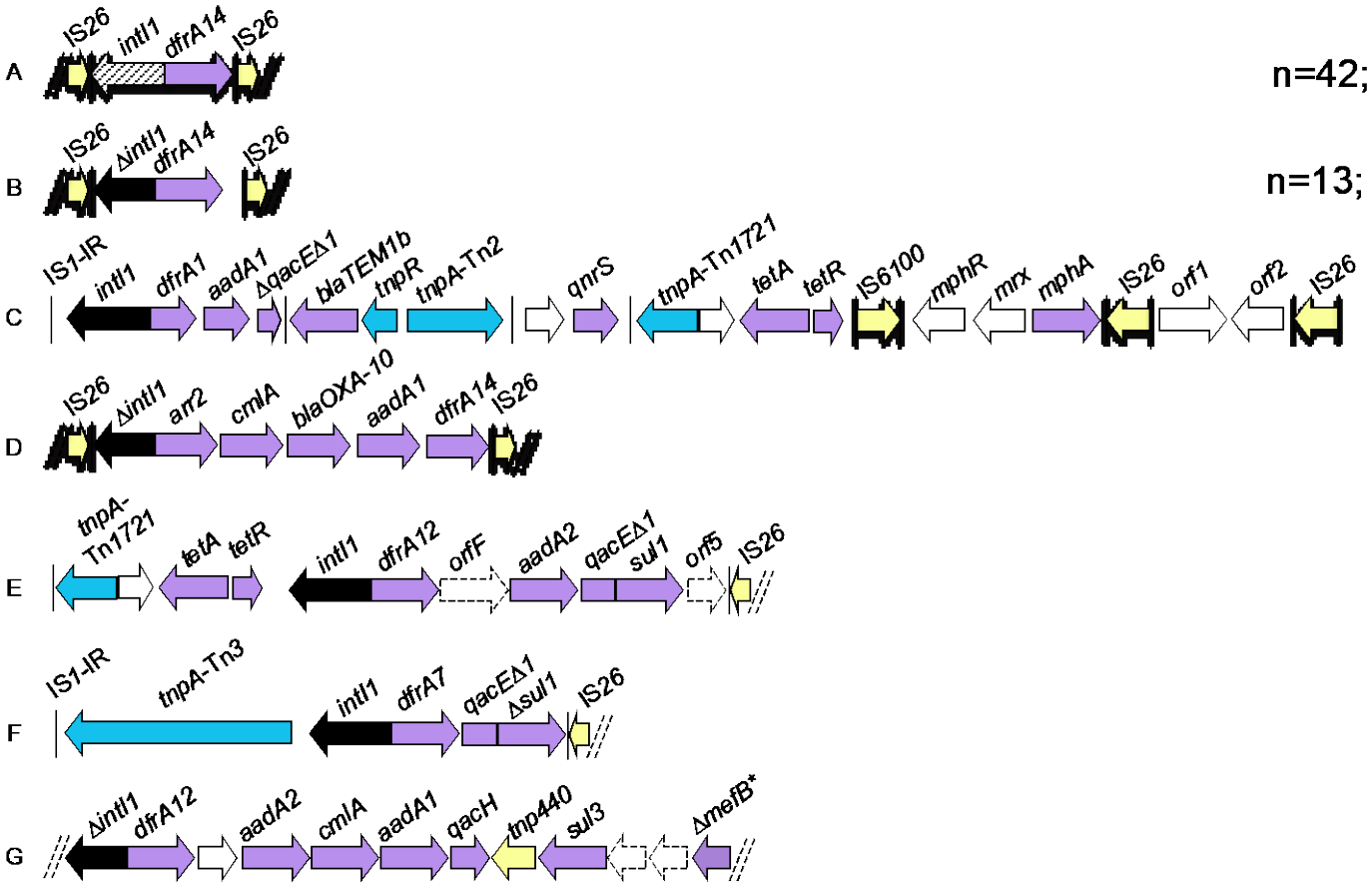


Reid, et al. 2020. Whole genome sequencing of *Escherichia coli* from store-bought produce. Front. Microbiol. 10:3050. doi.org/10.3389/fmicb.2019.03050.

ausgem

Whole Genome Sequencing of *E. coli* from produce

- Numerous class 1 integrons, carrying multiple AMR genes associated with IS elements such as IS*26*
- Carriage in multiple STs indicates horizontal gene transfer



Short read sequences did not allow the assembly of plasmids

ausgem

Exogenous capturing of transferable tetracycline resistance from produce into *E. coli* CV601

TET^{R} *E. coli* CV601 transconjugants	Sample source[a]	Inc groups[b]	*bla* genes	Resistance, integrase genes and IS[c]	Antibiotic resistance profile[d]
pBC1.1	Ci	P-1β^{f}	bla_{TEM}	*intI1, tet*(A), *merRTΔP, qacEΔ1,* IS*1071*	TE, AM, AMX, D
pBC1.3	Ci	P-1β^{f}	-	*intI1, tet*(A), *merRTΔP, qacEΔ1,* IS*1071*	TE, AM, AMX, D
pBC1.9	Ci	P-1β^{f}, FII^{l}	bla_{TEM}	*intI1, tet*(A), *merRTΔP, qacEΔ1,* IS*1071*	TE, AM, AMX, D
pBC1.12	Ci	P-1β^{f}, FII^{l}	-	*intI1, tet*(A), *merRTΔP, strA, qacEΔ1,* IS*1071*	TE, AM, AMX, D, S
pBC2.1	Ci	FIB^{l}	bla_{TEM}	*tet*(A), *sul1, qnrS*	TE, AM, AMX, D, CIP, NA, OFX, C
pBC2.2	Ci	FIB^{l}	bla_{TEM}	*tet*(A), *qnrS*	TE, AM, AMX, D, CIP, OFX
pBC2.3	Ci	FIB^{l}, I1^{f}	bla_{TEM}	*tet*(A), *qnrS*	TE, AM, AMX, D, CIP, OFX
pBC2.4	Ci	FIB^{l}	bla_{TEM}	*tet*(A), *sul2, qnrS*	TE, AM, AMX, D, CIP, NA, OFX
pBC2.6	Ci	FIB^{l}	bla_{TEM}	*tet*(A), *qnrS*	TE, AM, AMX, D, CIP, OFX, C
PBC2.8	Ci	FIB^{l}	bla_{TEM}	*tet*(A), *qnrS*	TE, AM, AMX, D, CIP, NA, OFX
pBC2.11	Ci	FII^{l}	bla_{TEM}	*tet*(A), *qnrS*	TE, AM, AMX, D, CIP, OFX
pBC2.15	Ci	I1^{fl}	bla_{TEM}	*tet*(A), *qnrS*	TE, AM, AMX, D, CIP, OFX
pBMS1	MS	FII^{l}	bla_{TEM}	*intI1, tet*(Q), *sul1, strA, merRTΔP*	TE, AM, AMX, D, TMP, C, S, SD
pBMS4	MS	FII^{l}	bla_{TEM}	*intI1, sul1, strA, merRTΔP,*	TE, AM, AMX, D, TMP, C, S, SD
pBA1	A	ND	bla_{TEM}	*tet*(A)	TE, AM, AMX, D, TMP, C, CIP
E. coli CV601 (R)	-	-	-	-	-

[a]: Ci: Cilantro; MS: Mixed salad; A: Arugula; [b]: l: detected by RT-PCR and PBRT; f: detected by RT-PCR; [c]: IS, Insertion Sequence.
[d]: C: Chloramphenicol; AM: Ampcillin; AMX: Amoxicillin; TE: Tetracycline; CIP: Ciprofloxacin; OFX: Ofloxacin; D: Doxycycline; NA: Nalidixic acid; TMP:Trimethoprim; SD: Sulfadiazine; ND: not detected.

Detection of IncF, I1, I2 plasmids and *intI1* and *tet*(A) from TC-DNA extracted from produce directly or after enrichment.

Produce	DNA		IncF		IncI1		IncI2		*intI1*		*tet*(A)	
			qPCR	Blot	qPCR	Blot	qPCR	Blot	qPCR	Blot	qPCR	Blot
Mixed salad	**Direct extraction**	0	-	-	-	-	-	+++	+	(+++)	-	(++)^{1*}
		7	-	-	-	-	-	+++	+	(+++)	-	(+++)^{2*}
	Enrichment	0	+	(+++)	+	(++)	+	+++	+	(+++)	+	(++)^{2*}
		7	+	(+++)	+	(++)	+	+++	+	(+++)	+	+++
Arugula	**Direct extraction**	0	-	-	-	-	-	(++)^{3*}	+	(++)	-	(+++)^{2*}
		7	-	-	-	-	-	(++)^{1*}	+	(++)	-	(+++)^{1*}
	Enrichment	0	+	(+++)	-	-	+	+++	+	(++)	+	+++
		7	+	(+++)	-	-	+	+++	+	(++)	+	+++
Cilantro	**Direct extraction**	0	-	-	-	-	-	-	(+)^{1*}	(++)	(+)^{1*}	(+++)^{3*}
		7	(+)^{1*}	(++)^{2*}	-	-	-	-	(+)^{3*}	(+++)	(+)^{2*}	(+++)^{3*}
	Enrichment	0	(+)^{2*}	(+++)^{2*}	(+)^{1*}	(++)^{2*}	-	-	(+)^{3*}	(+++)	(+)^{2*}	(+++)^{3*}
		7	(+)^{2*}	(+++)^{2*}	(+)^{2*}	(++)^{2*}	-	-	(+)^{3*}	(+++)^{3*}	(+)^{3*}	(+++)^{3*}

(*): Number of positive replicates, -: Not detected or no signal, +: positive (qPCR), (++): Medium signal; (+++): Strong signal.

DNA-based methods clearly less sensitive – need to be compared with enrichment methods

Blau et al., 2018 mBio

Re-use of treated waste water for irrigation or hydroponic plant growth

Important for the plant:

Stoichiometry of the nutrients, micro-nutrients and optimal pH; absence of phytotoxic compounds

Important for consumption of plants by humans and animals:

Quality of the treated waste water in view

- Microbiome composition
- Presence/absence of potentially human or animal pathogenic microorganisms and viruses
- Transferable multi antibiotic resistances
- Micropollutants (pharmaceuticals, metal and disinfectant compounds)
- Temporal dynamics but also the source of the waste water (hospital, food or antibiotic production)

HypoWave - Use of hydroponic systems for resource efficient water reuse in agriculture

Prof. Dr.-Ing. Thomas Dockhorn

Technische Universität Braunschweig

GEFÖRDERT VOM

Bundesministerium für Bildung und Forschung

Lettuce hydroponically grown in differently treated waste water or Hoagland solution

http://www.hypowave.de/fileadmin/user_upload/Ergebnisse/Vortr%C3%A4ge/IWA_HypoWave_Bliedung_V1.pdf

Quality – a short overview

- Heavy Metals
  - Most values were below the limits of quantification
  - Low concentrations in the shoot (e.g. Co and Hg were clearly below the limit values)
- Micropollutants
  - Detection of micropollutants in the roots (e.g. Acesulfam, **Carbamazepin, Diclofenac**)
  - Detection of some micropollutants in the shoot (e.g. Acesulfam, **Carbamazepin**)
  - Concentration depends on the treatment train
- Hygiene
  - *E.coli* detected in roots only of SBR‘s irrigation water
  - E.coli detected in shoots only in a single replicate (Hoagland Solution)
  - *Ps. aeruginosa* below detection limit
  - Similar CFU counts (also for coliforms) per run
  - Multi-resistant coliforms (*Enterobacter, Klebsiella, Citrobacter, E. coli*) mainly detected after enrichment

Conclusions and implication for food safety

- Via organic fertilizers and irrigation water bacteria carrying plasmid-localized ARGs are introduced together with pollutants (antibiotics, metal and disinfectant compounds or pesticides) into the agro-ecosystem

- Mobile genetic elements such as plasmids seem to be key for rapid adaptation of the bacterial hosts to the diverse micro-pollutants

- The rare microbiome of plants that carries transferable ARGs might proliferate under selective conditions and transfer resistances required to the human gut microbiota

- Direct-DNA based methods are often not sensitive enough to detect ARGs and MGEs present in the rare microbiome and are not suitable to determine resistance gene transferability and the genetic context (needs at least long reads)

- We propose that transferable resistome of produce might be a major link between the environments and humans

Thank you for your attention!

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