Extent and ecological significance of homologous recombination in *Xylella fastidiosa*

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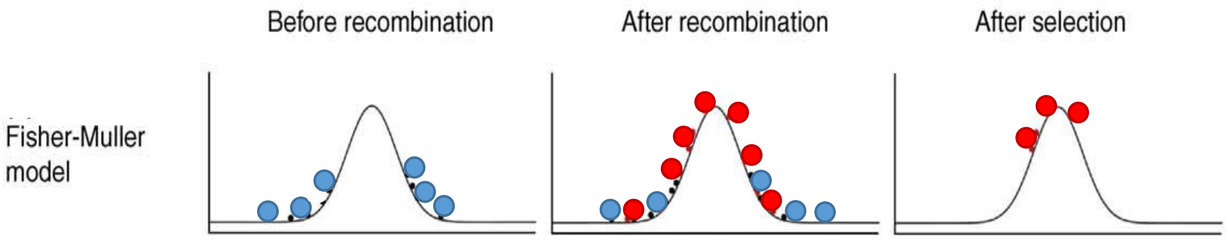
Generation of genetic variation

- **Point mutations**: DNA copying errors. Vertical inheritance.

- **Horizontal gene transfer (HGT)**: DNA acquired from other sources via **homologous recombination (HR)** ~sex in eukaryotes.

- **HR** increases genetic variation potentially **improving response to natural selection** (Vos, 2009. *Trends Microb.*).

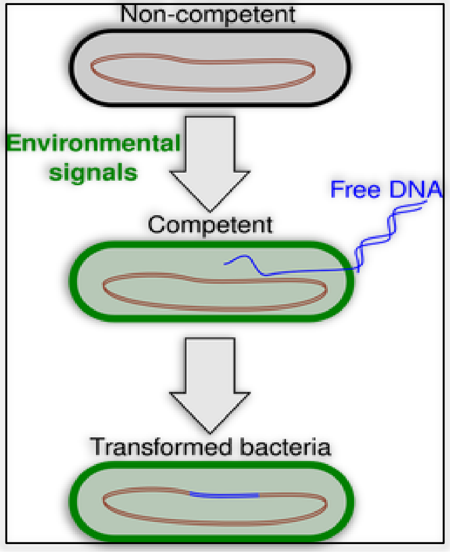


Fitness landscape

MLST studies *in **Xylella fastidiosa***: HGT more important than point mutations for genetic diversity (Scally et al., 2005).

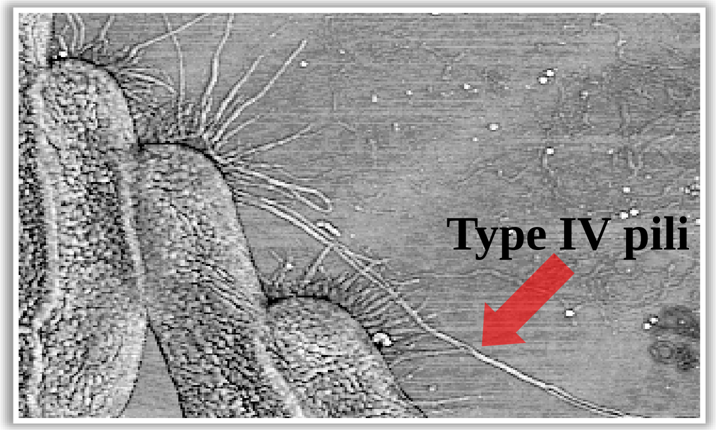
Natural competence/transformation

- **DNA** from dead cells or secreted by other live cells taken up from the **environment and recombined**.



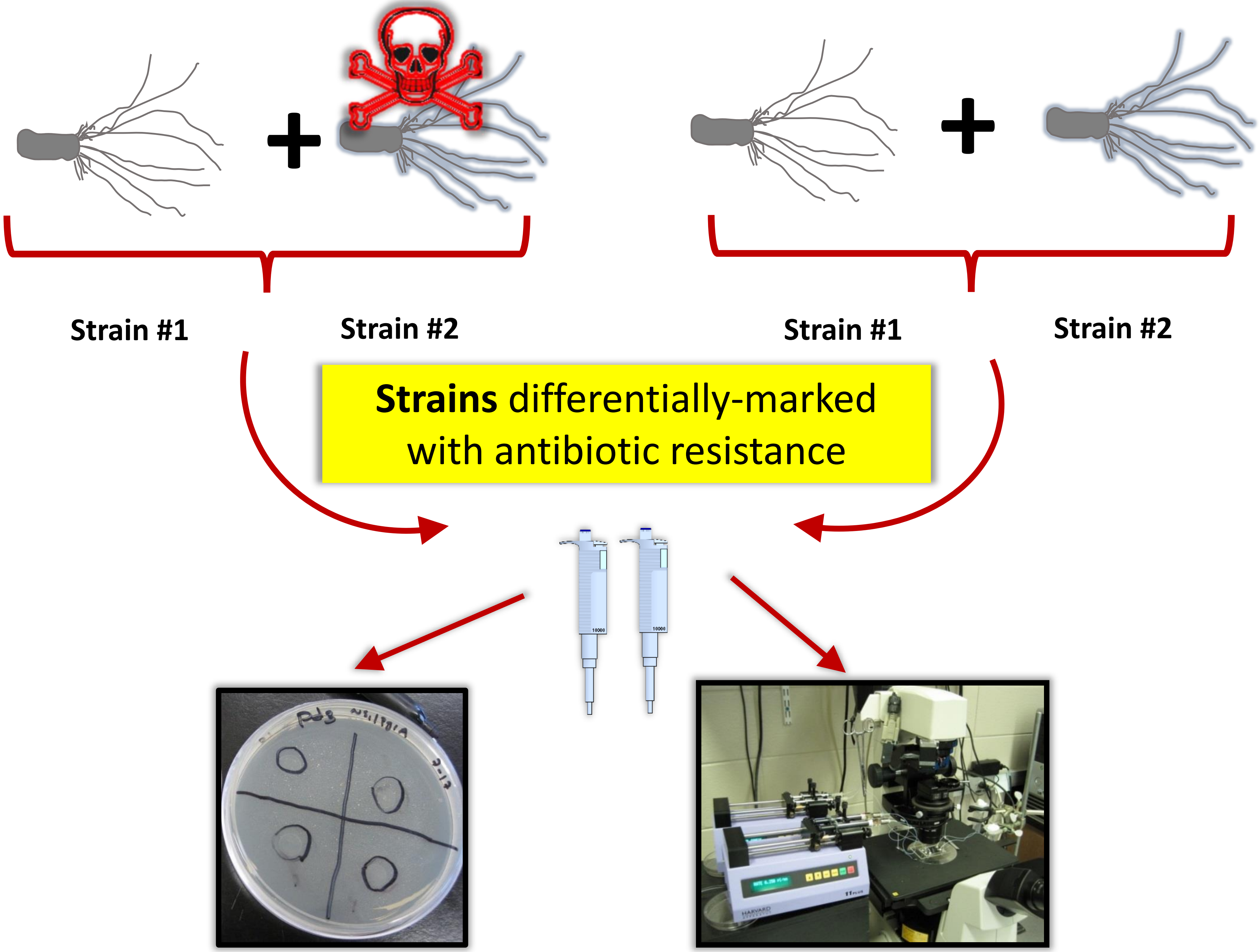
- Occurs in **highly adaptive human pathogens** and two plant pathogens.
- ***X. fastidiosa* is one of them**.

(Kung & Almeida 2011, 2014)



X. fastidiosa. Photo: Y. Meng; H Hoch.

Homologous recombination by natural transformation in vitro



Strain #1

Strain #2

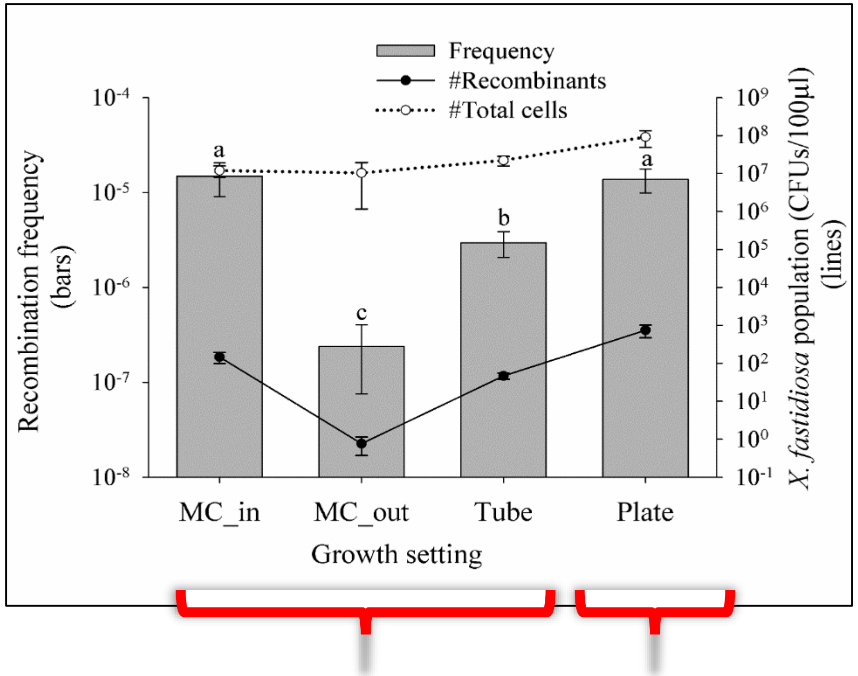
Strain #1

Strain #2

Strains differentially-marked with antibiotic resistance

Natural competence under flow conditions

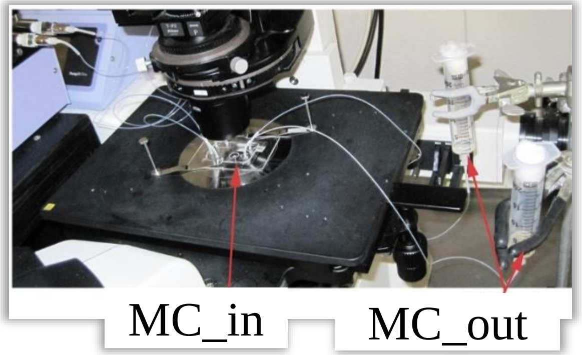
Flow conditions and supplemental **calcium** supported **highest** natural competence

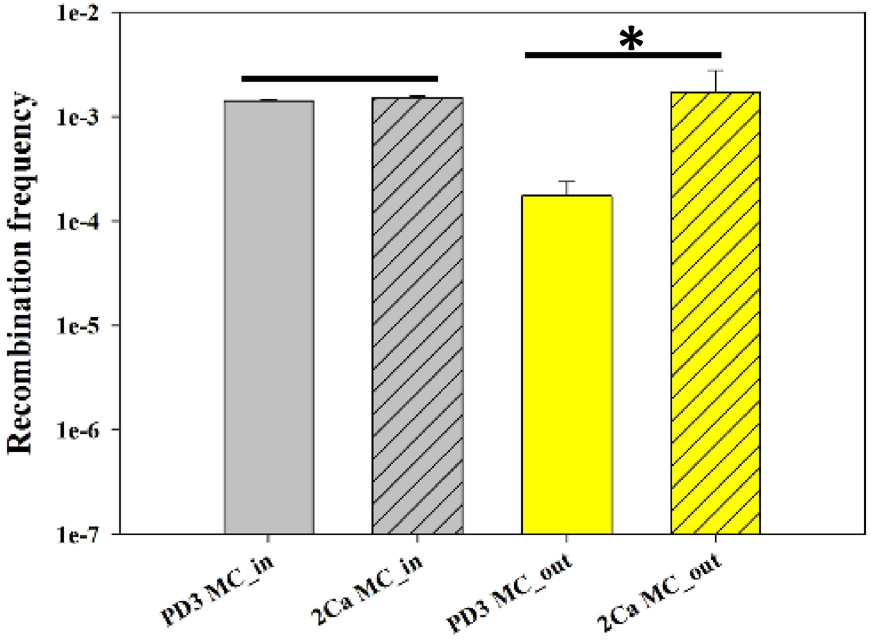


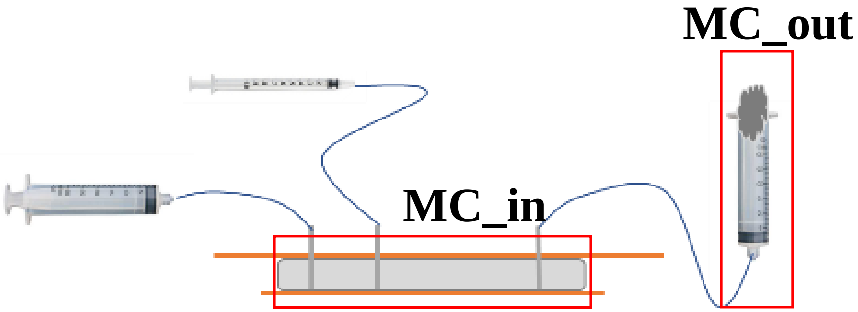
Liquid

Solid

Microfluidic Chambers (**MC**)







Kandel et al., 2016.
Appl. Env. Microb. 82:5269

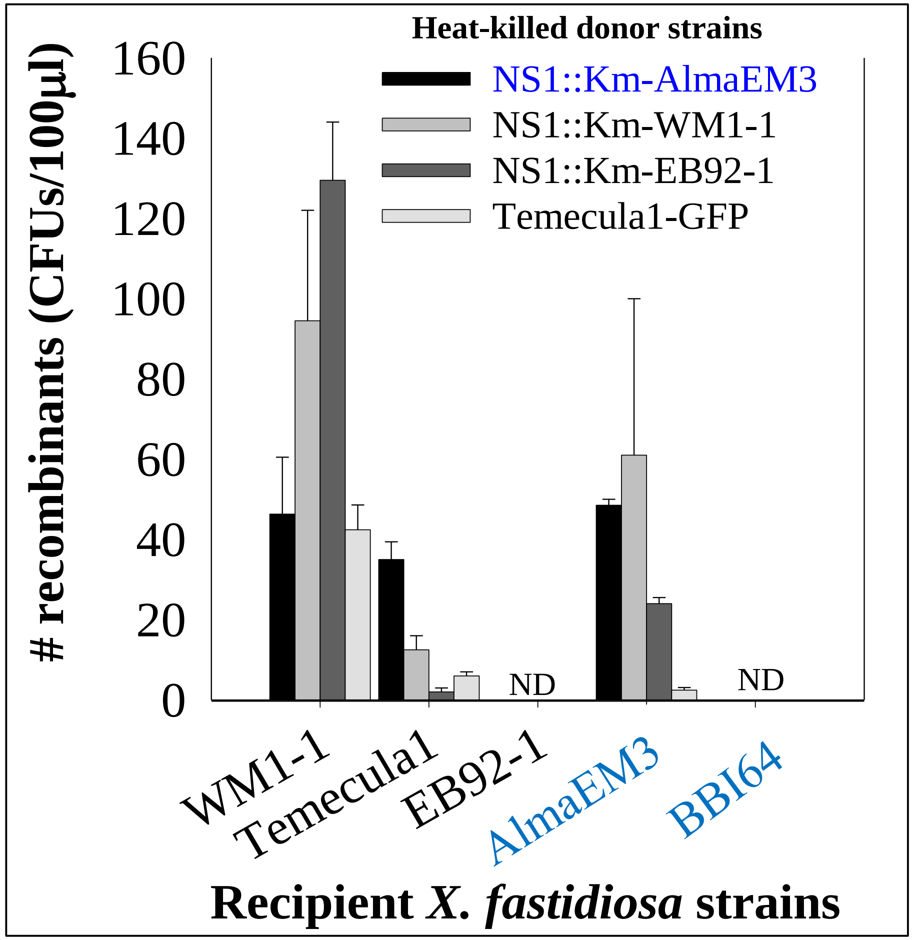
Chen & De La Fuente, *under review.*

Intra- and inter- subspecific recombination in *X. fastidiosa*

- Recombination occurred with **both live and live-dead** combinations of donor and recipients.
- Recombination **rates** are **variable depending on each strain**.

***X. f.* subsp. *fastidiosa*: WM1-1, EB92-1, Temecula**

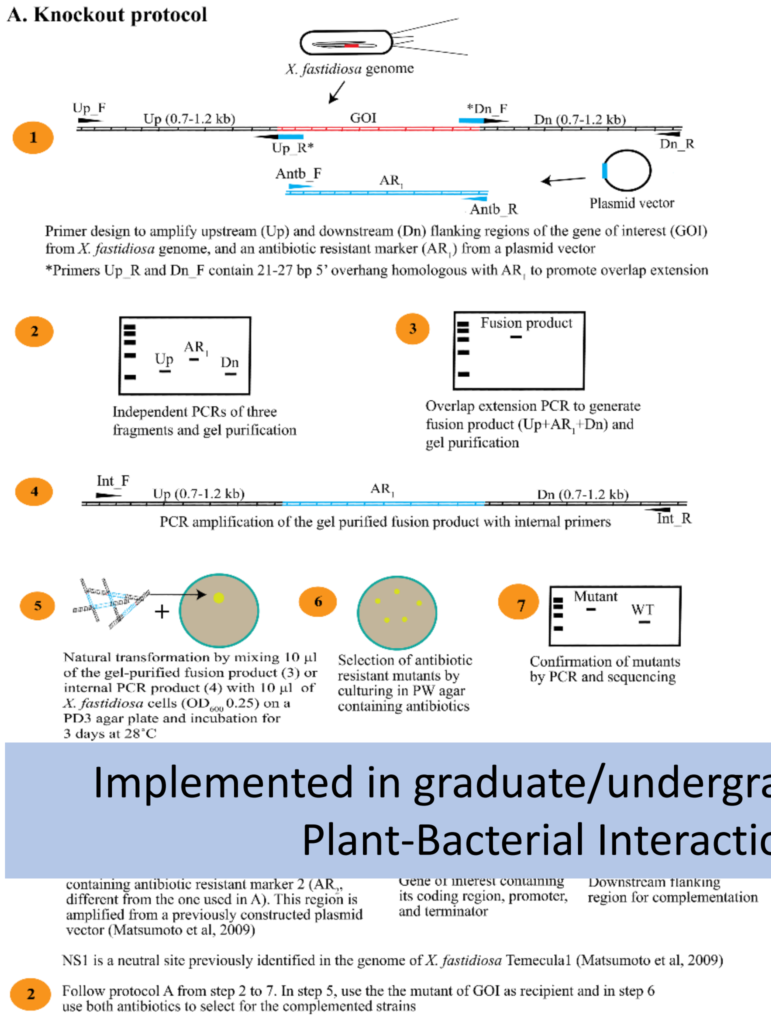
***X. f.* subsp. *multiplex*: AlmaEm3, BBI64**



Kandel et al., 2017.
MPMI 30:589

Short protocol for *X. fastidiosa* mutagenesis: natural transformation & overlap PCR

More than 50 mutants generated < 2 years



A. Knockout protocol

Primer design to amplify upstream (Up) and downstream (Dn) flanking regions of the gene of interest (GOI) from *X. fastidiosa* genome, and an antibiotic resistant marker (AR_1) from a plasmid vector

*Primers Up_R and Dn_F contain 21-27 bp 5' overhang homologous with AR_1 to promote overlap extension

Independent PCRs of three fragments and gel purification

Overlap extension PCR to generate fusion product ($Up+AR_1+Dn$) and gel purification

PCR amplification of the gel purified fusion product with internal primers

Natural transformation by mixing 10 µl of the gel-purified fusion product (3) or internal PCR product (4) with 10 µl of *X. fastidiosa* cells (OD_{600} 0.25) on a PD3 agar plate and incubation for 3 days at 28°C

Selection of antibiotic resistant mutants by culturing in PW agar containing antibiotics

Confirmation of mutants by PCR and sequencing

containing antibiotic resistant marker 2 (AR_2, different from the one used in A). This region is amplified from a previously constructed plasmid vector (Matsumoto et al, 2009)

Gene of interest containing its coding region, promoter, and terminator

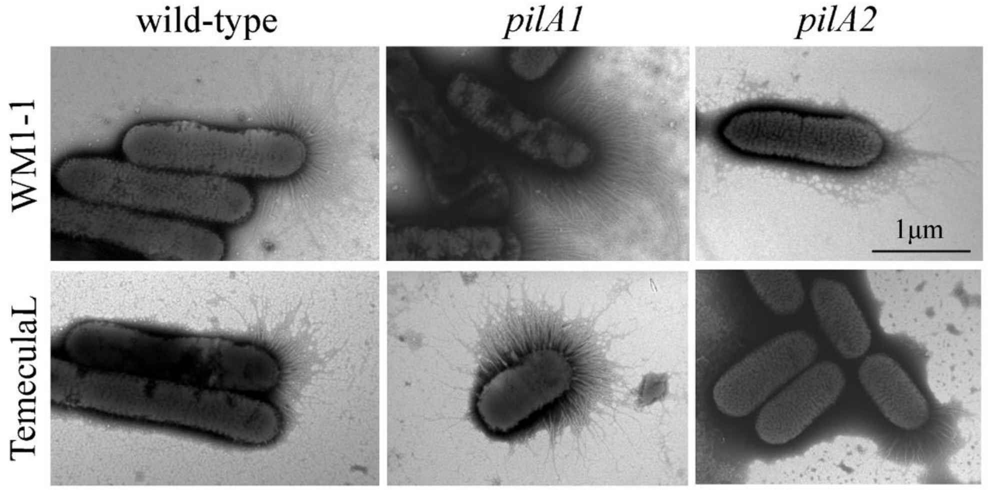
Downstream flanking region for complementation

NS1 is a neutral site previously identified in the genome of *X. fastidiosa* Temecula1 (Matsumoto et al, 2009)

Follow protocol A from step 2 to 7. In step 5, use the the mutant of GOI as recipient and in step 6 use both antibiotics to select for the complemented strains

Implemented in graduate/undergraduate course
Plant-Bacterial Interactions

Pilin (*pilA*) paralogs have different functions



Kandel, Chen & De La Fuente. 2018.
Appl. Env. Microb. 84:doi:10.1128/AEM.01167-18.

Genome sequences of experimentally-generated recombinants

Live

Dead

Selection of **recombinants**: acquisition of **antibiotic resistance**

TemeculaL + AlmaEM3(NS1::Km^R)

TemL(Alma) Rec 1 [Km^R]

TemL(Alma) Rec 2 [Km^R]

WM1-1 + KLN59.3 (GFP) (Temecula1) [Km^R]

WM1-1(GFP) Rec 1 [Km^R]

WM1-1(GFP) Rec 2 [Km^R]

NS1-Cm^R (Temecula L) + pglA-Km^R (Fetzer)

TemL(NS1pglA) Rec 1 [Cm^R Km^R]

Kandel, …, & De La Fuente. 2017. *MPMI 30:589.*

Extent of recombination in vitro

Inter-subspecific recombination (IHR)

TemeculaL + AlmaEM3(NS1::KmR)

IHR **only flanking** Km marker cassette: 10kb, 3.5kb.

Intra-subspecific recombination

WM1-1 + KLN59.3 (GFP) (Temecula1) [Km^R]

HR **flanking** GFP marker **AND away**: 10.5kb, 6.1kb.

NS1-Cm^R (Temecula L) + pglA-Km^R (Fetzer)

HR **flanking** Km cassette **AND away**: 0.3kb, 1.5kb, 3 kb.

BratNextGen (within subspecies) and fastGEAR (between subspecies)

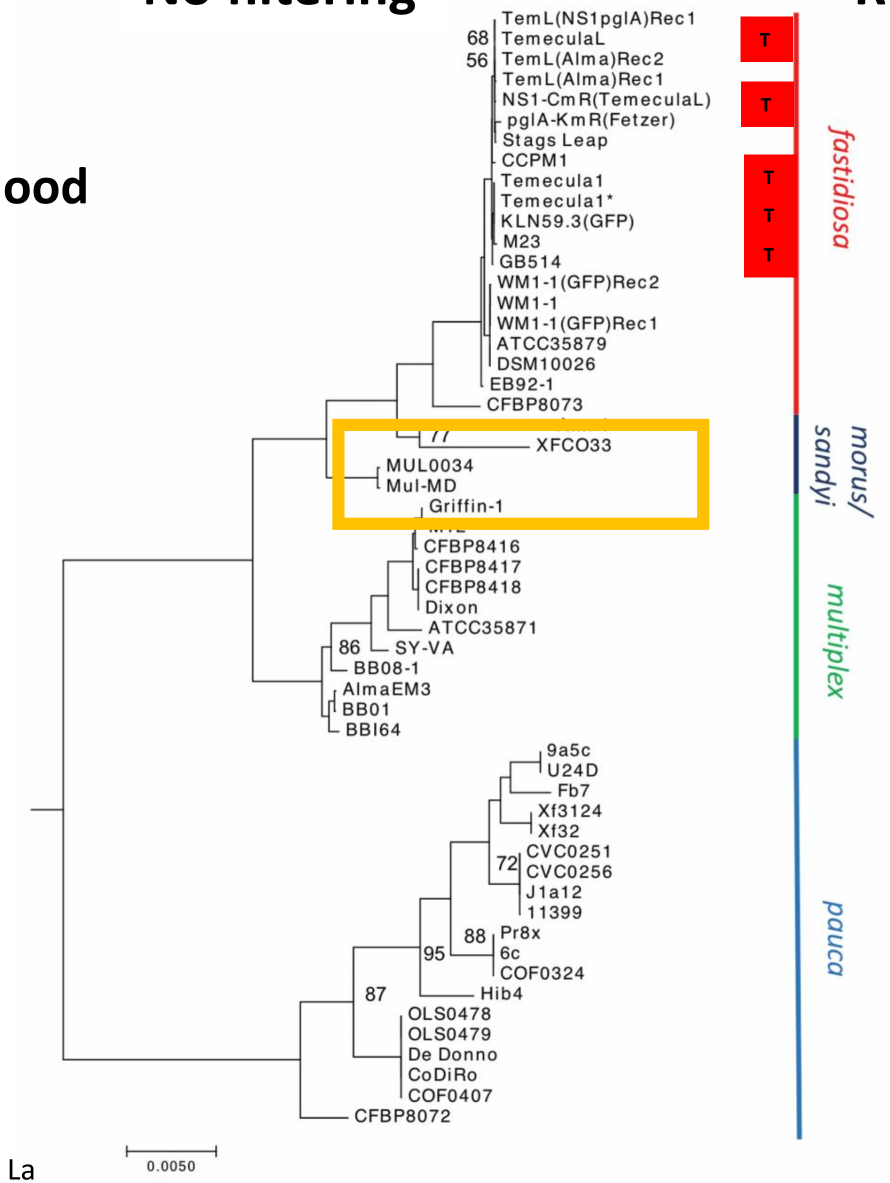
Recombination in nature

Population structure of *X. fastidiosa*

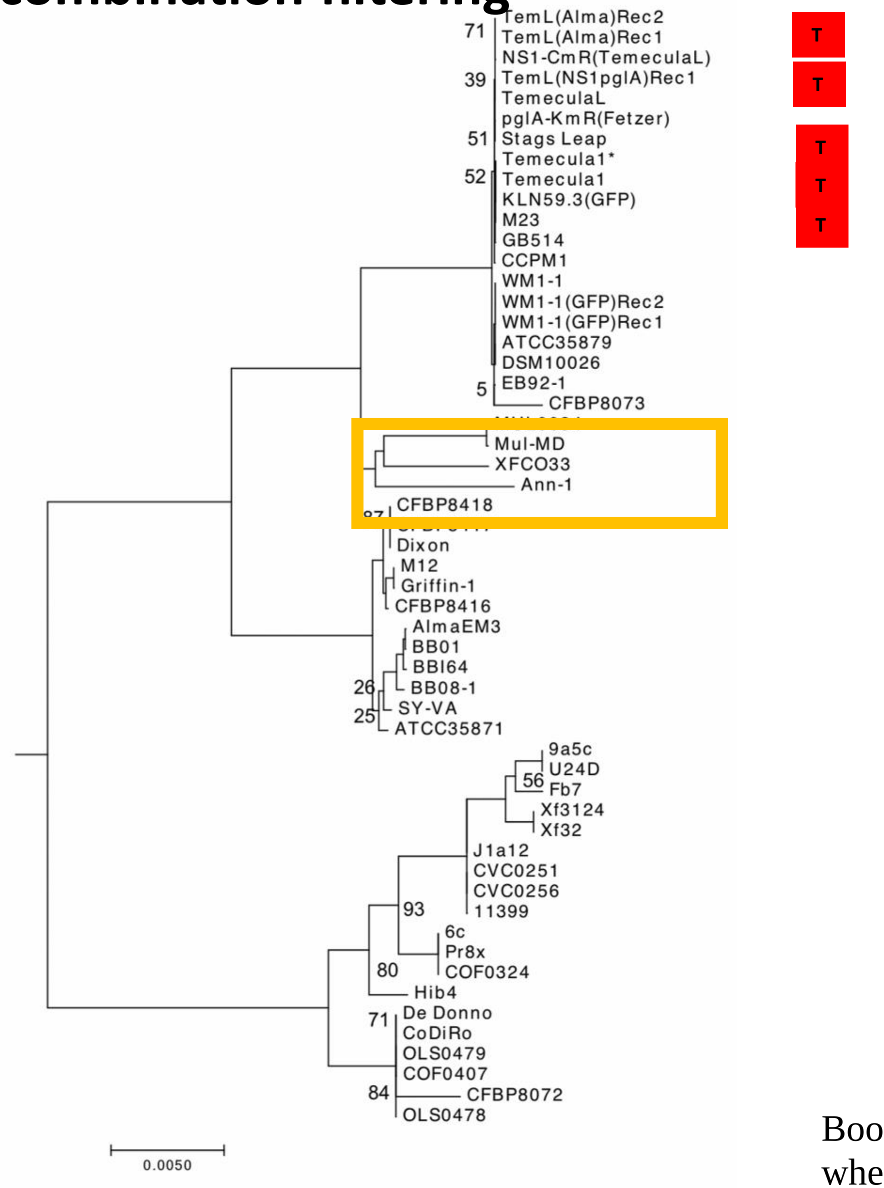
Maximum likelihood phylogeny

Core genome:
55 strains
1.79MB
Mauve

No filtering



Recombination filtering

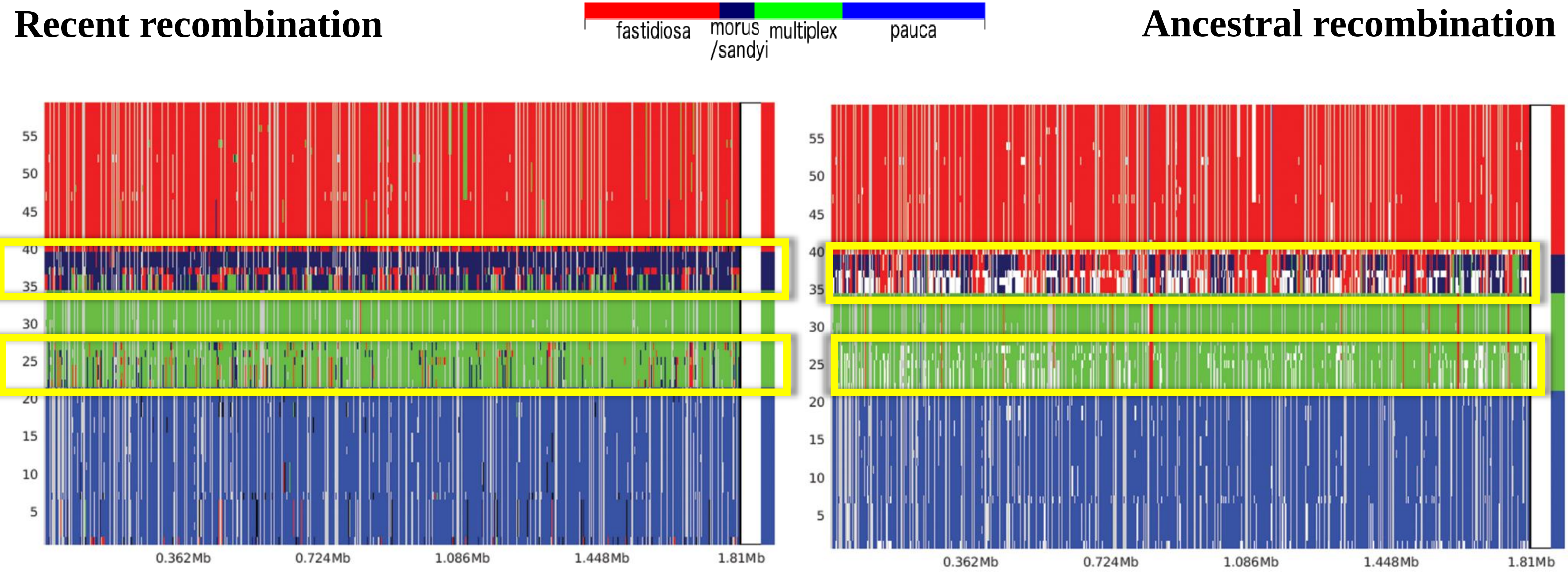


Temecula1?

Bootstrap >98% except where indicated.

Potnis, Kandel, Merfa, …, & De La Fuente. 2019. *The ISME J.* 13:2319.

Interlineage recombination events in *X. fastidiosa* wild-type genomes



Recent recombination

Ancestral recombination

Potnis, Kandel, Merfa, …, & De La Fuente. 2019. *The ISME J. 13:2319.*

fastGEAR

Extent of recent inter-subspecies recombination in nature

Variable among strains

	Total length recombinant region (kb)	Genome (%)	Recombination events (#)
subsp. *pauca*	~10 kb – 63 kb	~0.4 – 2.5 %	15-131
subsp. *fastidiosa*	~15 kb – 56 kb	~0.6 – 2.3 %	4-26
susbp. *multiplex*	~0.5 kb – 310 kb	~0.02 – 12 %	1-232
susbp. *morus/sandyi*	~31 kb – 679 kb	~1.2 – 27 %	23-232

Most intercepted strains: exception

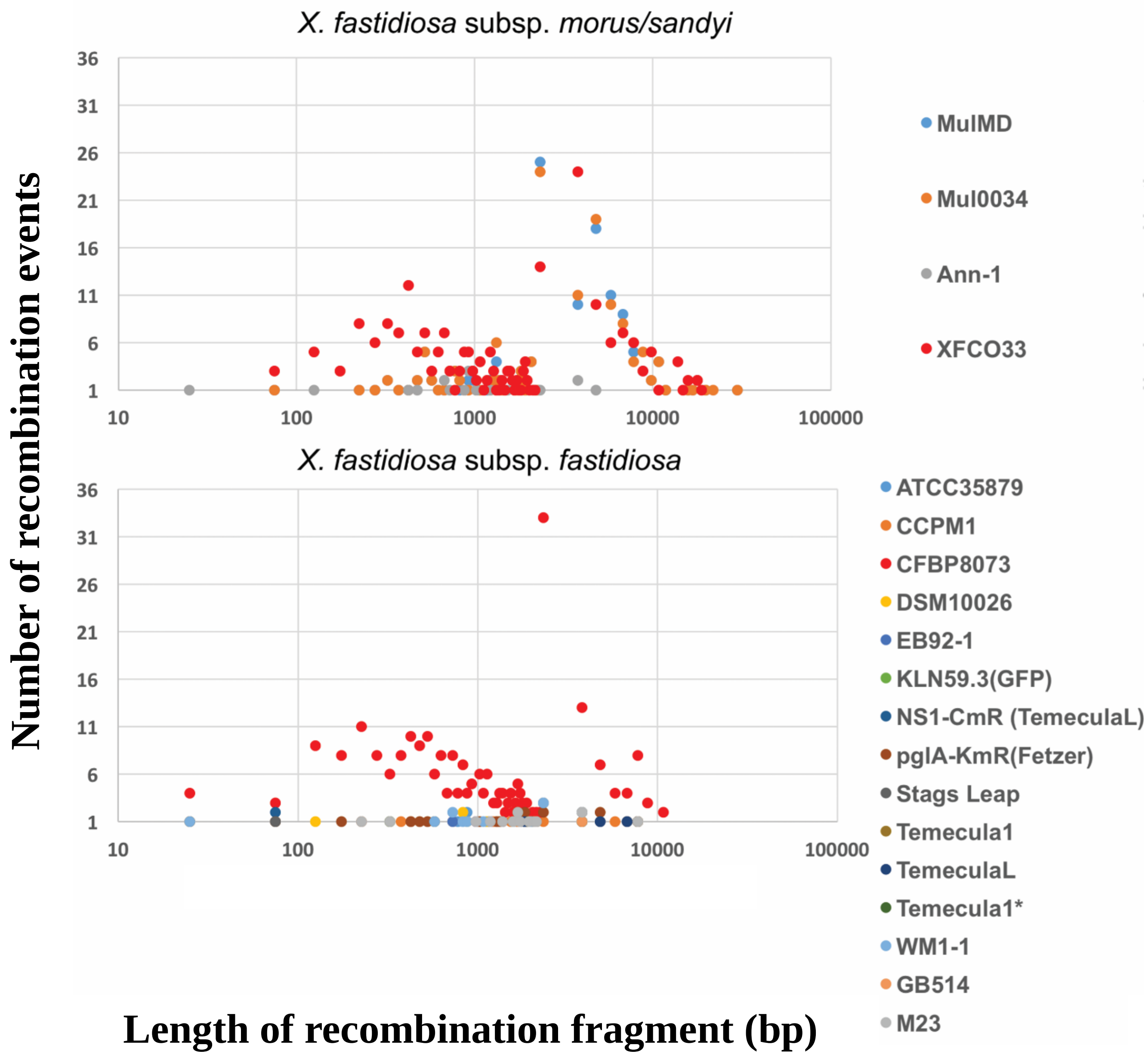
Extent of recent inter-subspecies recombination in strains from intercepted plants

	Strain	Sequence Type (ST)	Interception (country)	Origin (host/country)	Total length recombinant region (kb)	Genome (%)	Recombination events (#)
subsp. *pauca*	**CFBP8072[1]**	ST 74	France	Coffee/Ecuador	250 kb	10%	376
	PD7202[2]	ST 53	Netherlands	Coffee/Costa Rica	35kb	1.4%	49
	PD7211[2]	ST 73	Netherlands	Coffee/Costa Rica	71kb	3%	139
subsp. *fastidiosa*	**CFBP8073[1]**	ST 75	France	Coffee/Mexico	495 kb	20%	277
susbp. *sandyi*	**XFCO33[3]**	ST 72	Italy	Coffee/Costa Rica	671 kb	27%	232

[1]Jacques et al., 2016 *AEM*; [2]Bergsma-Vlami et al., 2017 *Plant Pathology*; [3]Giampetruzzi et al., 2015 *Genome Ann.*

Length and number of recombinant fragments

Intercepted strains ●



Potnis, Kandel, Merfa, …, & De La Fuente. 2019. *The ISME J. 13:2319.*

Recombination ‘hot spots’

Recombination fragments shared in vitro and WT: hotspots?

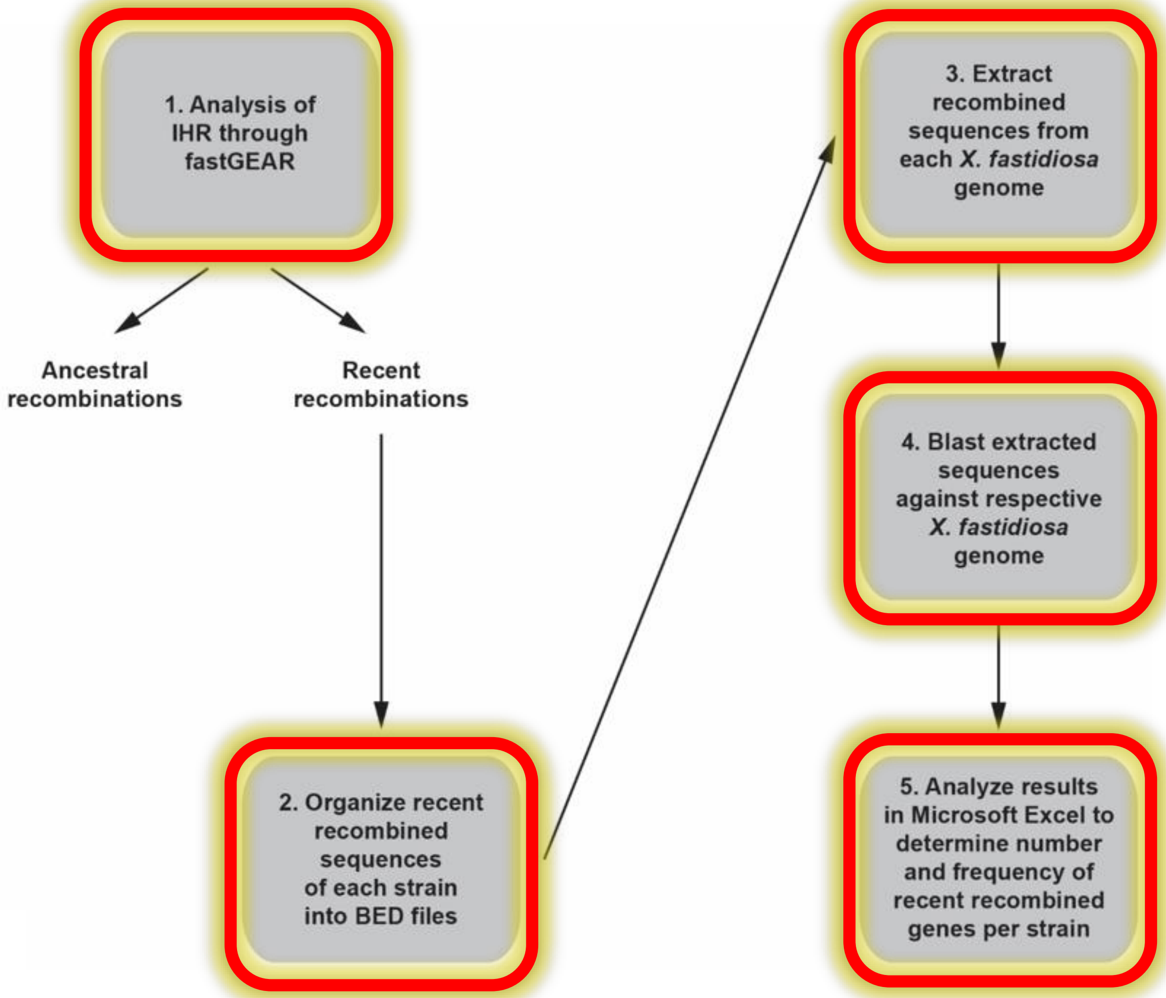
Recombined gene (recent)	In vitro mutant	Wild-type subspecies (# recombination events):				Function	Reference
		morus/sandyi	*multiplex*	*fastidiosa*	*pauca*		
Lipase/alpha-beta hydrolase (PD1702)	WM1-1 (GFP) Rec1	2	ND	ND	11	Xf: Homolog (PD1703) identified as pathogenicity effector	Zhang et al., 2015*
Putative Ctpa-like serine protease /peptidase S41 (PD0948)	WM1-1 (GFP) Rec1	1	15	1	ND	Associated with virulence of other bacterial species	Carroll et al., 2014.
Putative Ctpa-like serine protease/ C-terminal processing protease CtpA/Prc (PD0949)	TemL(NS1-pgla) Rec 1	1	15	1	ND	Associated with virulence of other bacterial species	Carroll et al., 2014.
Extracellular serine protease (PD0950)	TemL(NS1-pgla) Rec 1	2	ND	ND	11	Xf: Serine protease PD0956 identified as pathogenicity effector	Zhang et al., 2015*

*Genes identified by comparison **virulent** (Temecula1) vs. **avirulent** (EB92-1) strain.

ND: not detected.

Function of genes under recombination

Pipeline: number and frequency of recent recombination events of each gene



1. Analysis of IHR through fastGEAR

Ancestral recombinations

Recent recombinations

2. Organize recent recombined sequences of each strain into BED files

3. Extract recombined sequences from each *X. fastidiosa* genome

4. Blast extracted sequences against respective *X. fastidiosa* genome

5. Analyze results in Microsoft Excel to determine number and frequency of recent recombined genes per strain

Potnis, Kandel, Merfa, …, & De La Fuente. 2019. *The ISME J. 13:2319.*

Recent intersubspecific recombined genes analyzed

- Analysis of functional significance of **1,026 annotated genes** (~40% of the *X. fastidiosa* genome), that were identified in recombinant regions.

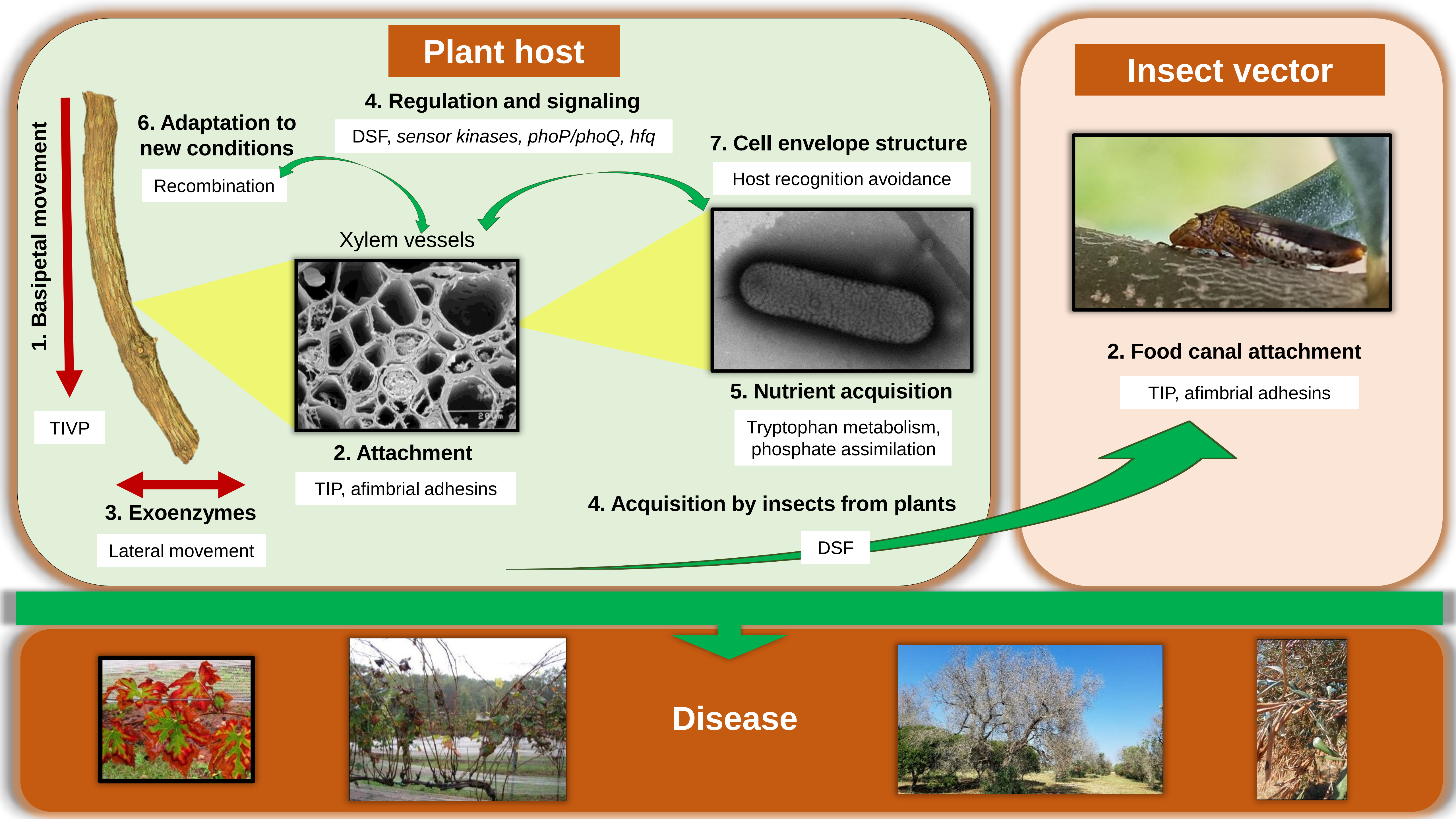
- Selected for discussion: recently-recombined annotated loci that were included among the **top 10%** in terms of frequency of recombination events (**105 genes** with **19 recombination events** or higher).

Most frequently recombined genes

Gene (most frequent to least frequent)	Number of recombination events
Hypothetical protein	
Vitamin B12 transporter BtuB	**B12 human pathogens**: regulates gene expression, enzyme activity, abundance microorganisms, virulence, biofilm.
Sensory/regulatory protein RpfC	**DSF Xf**: quorum sensing, biofilm, movement.
Toxin RTX-I translocation ATP-binding protein	71
3-oxoacyl-[acyl-carrier-protein] reductase FabG	62
Sulfate/thiosulfate import ATP-binding protein CysA	54
Energy-dependent translational throttle protein EttA	52
Lipopolysaccharide export system ATP-binding protein LptB	52
sn-glycerol-3-phosphate import ATP-binding protein UgpC	52
ABC transporter ATP-binding protein uup	51

Recent recombined genes: functional categories

Recent recombined genes (category/molecular component)		Ecological role
1. Movement	Type IV pili genes	**Xf**: Plant colonization via twitching motility; virulence determinant.
2. Attachment	Type I pili	**Xf**: Cell-cell aggregation, attachment to surfaces and biofilm formation; important for colonization of plant and insect, virulence determinant.
	Afimbrial adhesin genes	
3. Exoenzymes	TIISS genes	**Xf**: Hydrolytic enzymes, degrading plant structural components, colonization.
	Xylan 1,4-xylosidase	
4. Regulation and signaling	DSF	**Xf**: Quorum sensing, switch plant-insect environments.
	Sensor kinases	Two-component signal transduction, external stimuli response, environment adaptation.
	phoP/phoQ	**Xf**: Essential for survival in plant host.
	Vitamin B12 transporter BtuB	Regulatory system, affects virulence and biofilm formation.
	RNA binding Hfq	Small RNAs chaperone, regulates virulence and stress adaptation.
5. Nutrient acquisition	Tryptophan metabolism	Important for interaction host-microflora in humans.
	Phosphate assimilation	**Xf**: Indirect evidence suggests phosphorus metabolism important for plant colonization.
6. Adaptation to new conditions	Recombination genes	**Xf**: Acquisition of new genomic information, evolution.
	tRNA biosynthesis / modification genes	Optimization of translation, adaptation to changes.
7. Cell envelope structure	Peptidoglycan biosynthesis genes	Plant host recognition, bacterial growth.
	Lipopolysaccharide genes	**Xf**: O-antigen - delay initial recognition by plant innate immune system.
	3-Oxoacyl [acyl-carrier-proteins]	Fatty acid metabolism, influence membrane structure and quorum sensing signaling.



Plant host

1. Basipetal movement

TIVP

3. Exoenzymes

Lateral movement

6. Adaptation to new conditions

Recombination

4. Regulation and signaling

DSF, *sensor kinases, phoP/phoQ, hfq*

Xylem vessels

2. Attachment

TIP, afimbrial adhesins

7. Cell envelope structure

Host recognition avoidance

5. Nutrient acquisition

Tryptophan metabolism, phosphate assimilation

4. Acquisition by insects from plants

DSF

Insect vector

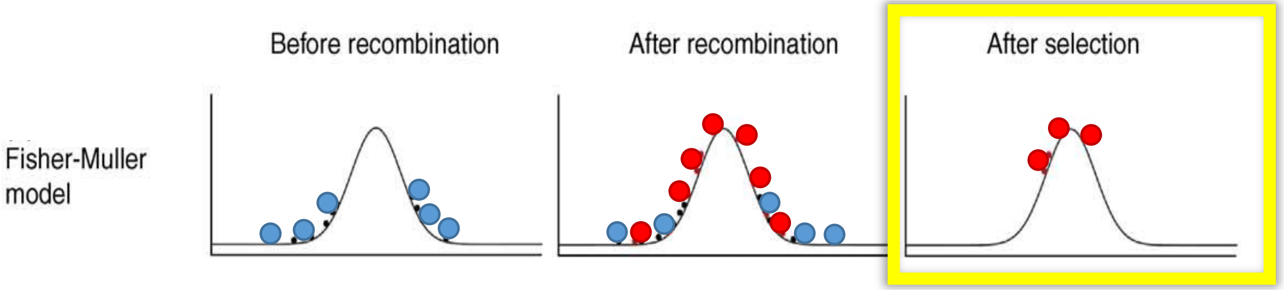
2. Food canal attachment

TIP, afimbrial adhesins

Disease

Conclusions: Homologous recombination

- Location of recombinogenic regions **in vitro** is **variable**, and occurs **away** from the antibiotic resistance cassette.
- Homologous recombination in **wild-type** genomes **influences phylogenetic relationships**.



- Many **genes under recombination** are important for **bacterial fitness, virulence** and **ecological adaptation**.

Hypothesis: homologous recombination improves response to natural selection (viz.; **increase fitness**) of *Xylella fastidiosa.*

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efsa
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