



YANN REYNAUD

Searching for genetic markers of virulence in *Vibrio nigripulchritudo*, a *Litopenaeus stylirostris* shrimp pathogen



Shrimp aquaculture in New Caledonia

An unique shrimp specie *L. stylirostris*

2 bacterial diseases

1) Syndrome 93

- Caused by *Vibrio penaeicida*
- Since 1993 in all the shrimp farms of the territory
- Lead to stop the production during the cold season

Other bacterial species were isolated without being involved in the disease: V. nigripulchritudo

2) Summer Syndrome

- Caused by *Vibrio nigripulchritudo*
- First observed in 1997 in one farm...
...apparently spreading to other farms

Objectives

- Characterize the mechanisms of virulence implicated in the pathogenesis of *V. nigripulchritudo*



- Development of relevant diagnostic tools of the summer syndrome
- Ways to control the disease...prophylactic or curative methods

Methods

- 1) Collection of *V. nigripulchritudo* strains in different context: Summer Syndrome, Syndrome 93, opportunistic mortalities, samples without mortalities
- 2) Experimental infection: definition of different virulence status
- 3) Genotyping by MLST and AP-PCR
- 4) Identification genetics markers of virulence by SSH and macroarray

Methods

1) Collection of *V. nigrripulchritudo* strains in different context: Summer Syndrome, Syndrome 93, opportunistic mortalities (Goarant et al., 2005)

2) Experimental infection: definition of different virulence status

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3 virulence status of *V. nigripulchritudo* by experimental infection

intramuscular injection of a bacterial suspension (500 CFU/shrimp)

- Non virulent (Vir-)
final survival rate after 72h: >80%
- Moderately virulent (Vir+/-)
20% < SR < 80%
- highly virulent (Vir+)
SR < 20%



Methods

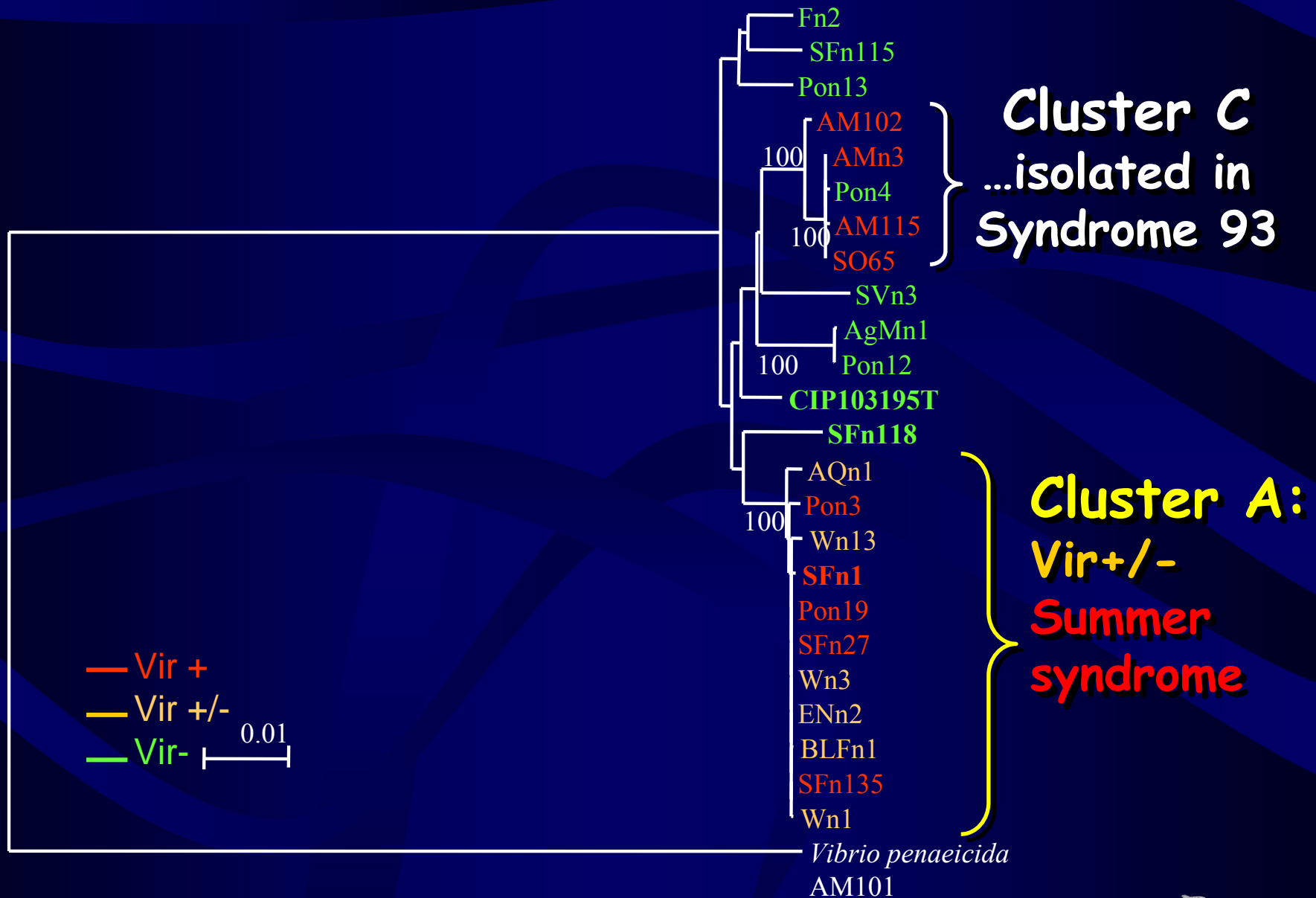
1) Collection of *V. nigripulchritudo* strains in different context: Summer Syndrome, Syndrome 93, opportunistic mortalities

2) Experimental infection: definition of different virulence status

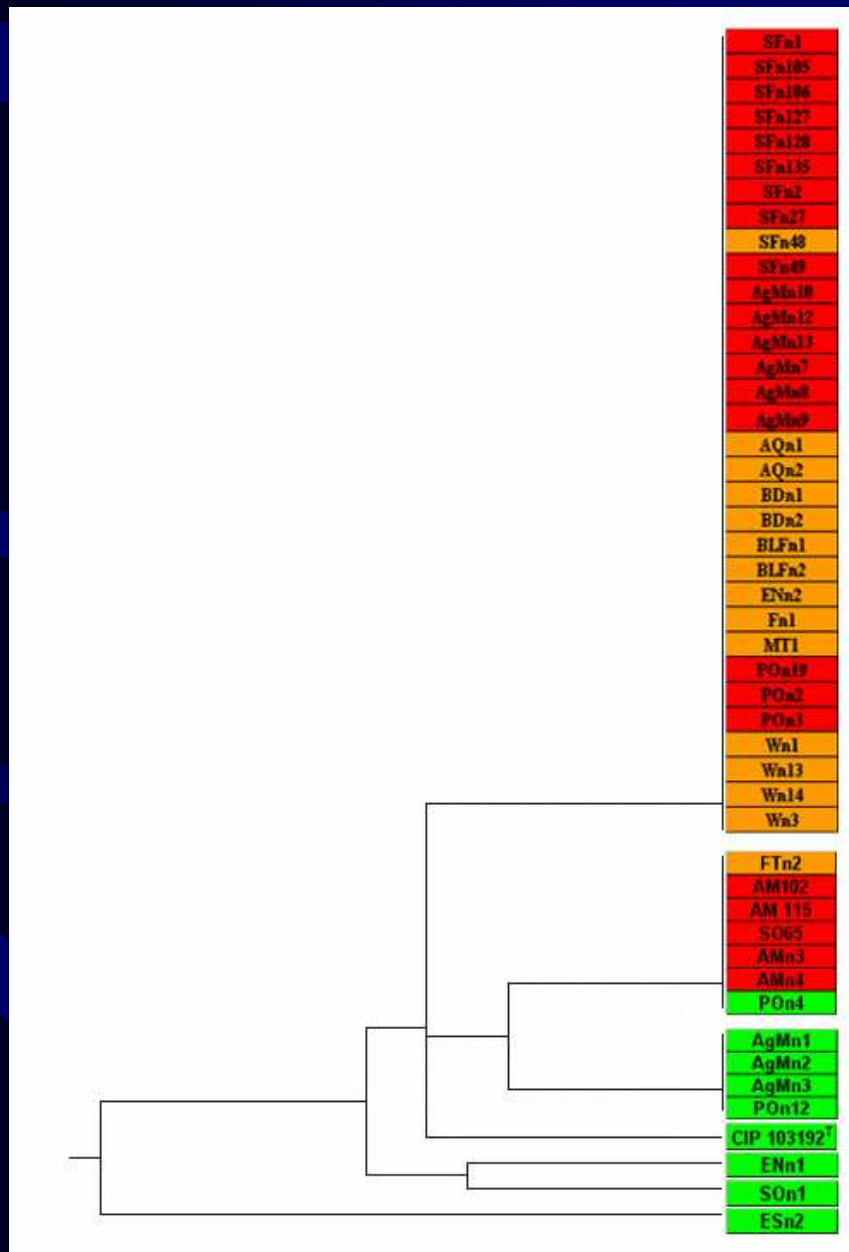
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MLST (3630 BP)



AP PCR results



Cluster A

Cluster C

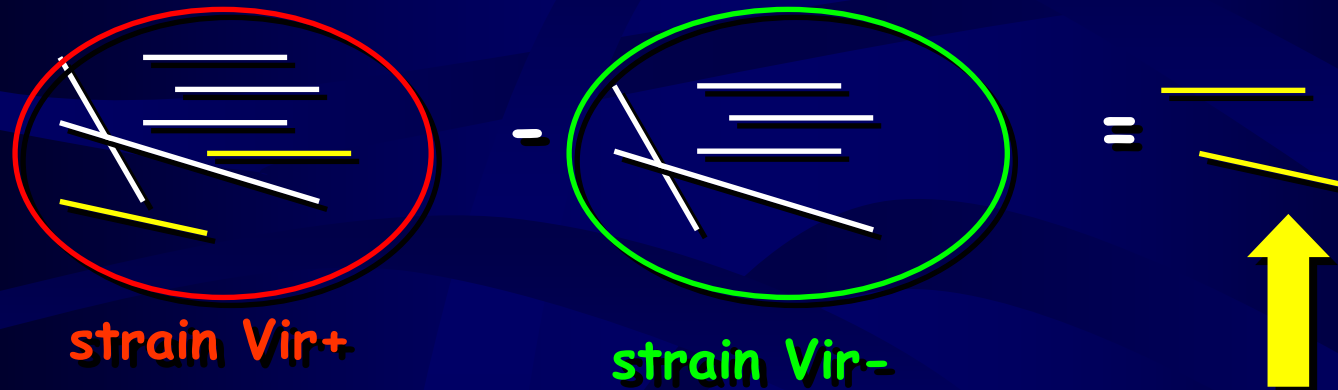
Congruent results with
MLST (goarant et al., 2006)

Methods

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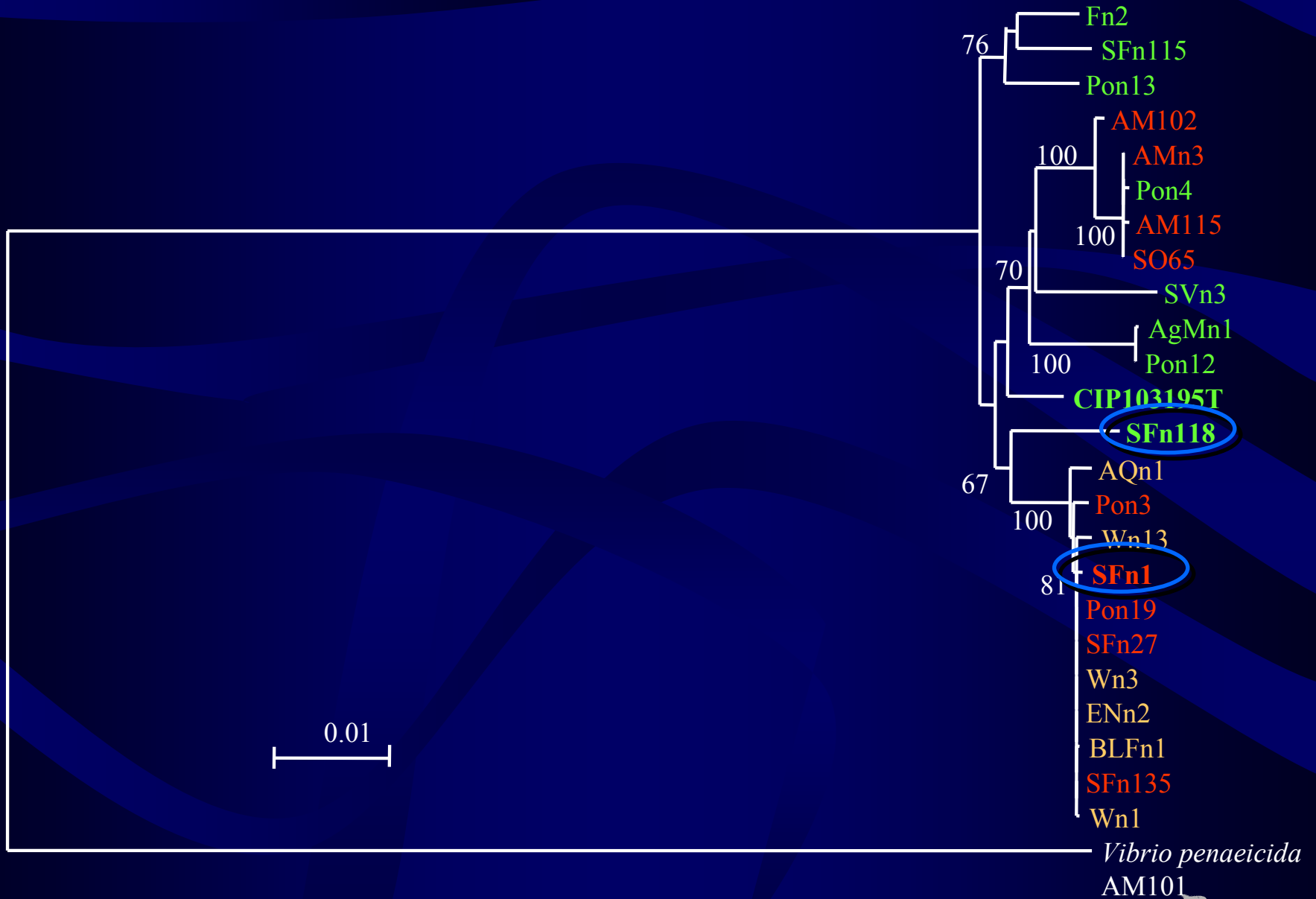
SSH (Suppression Subtractive Hybridization)

the method briefly...



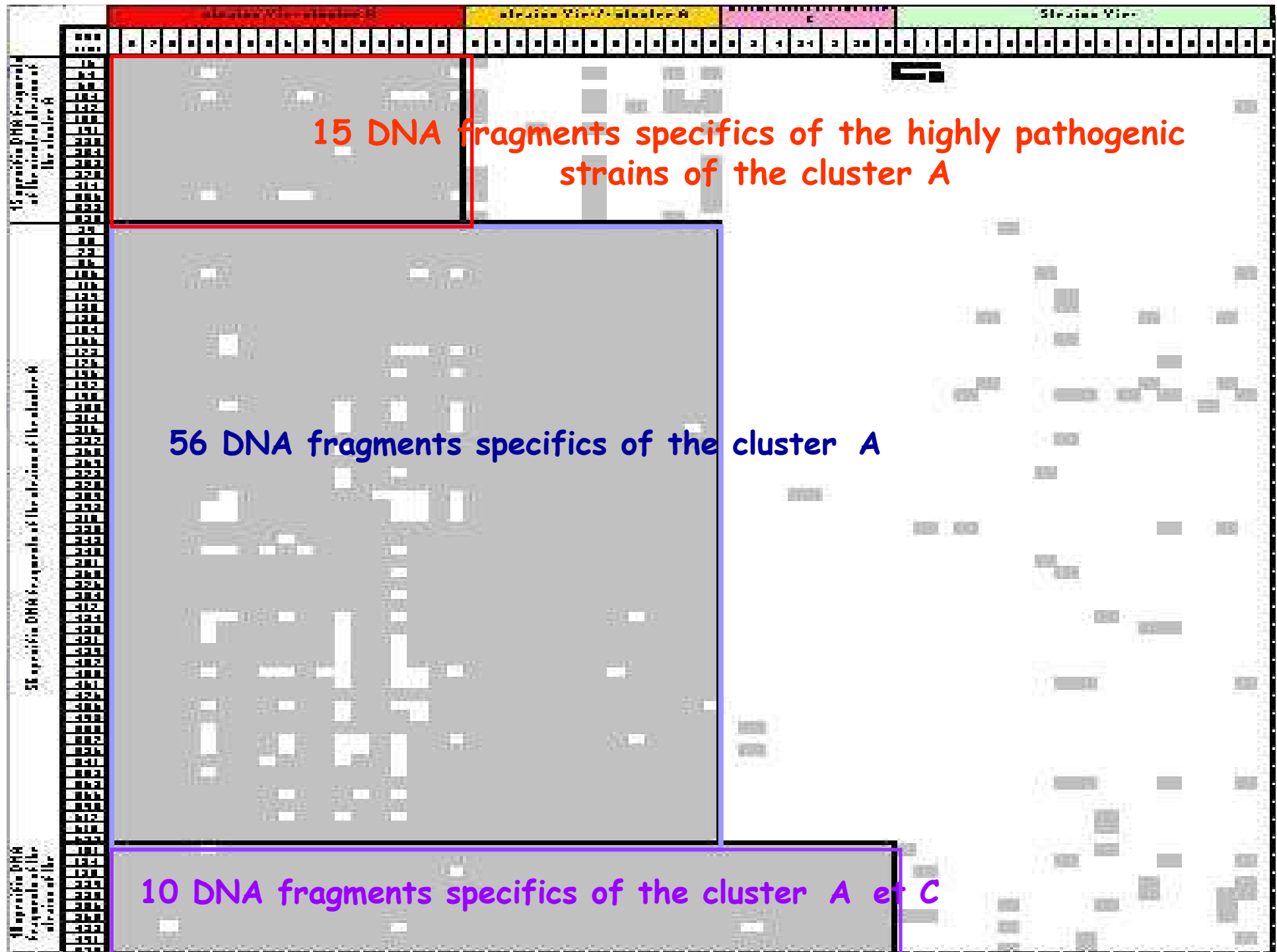
Construction of SSH library: specific DNA fragments of the virulent strain

SFn1 the tester and SFn118 the driver



SSH approach

- DNA extraction of tester and driver
- Construction of the library: 1112 clones
- Screening of the real SFn1 specific DNA fragments
- Sequencing
- Blast on genbank: 511 DNA fragments
- Clustering of these DNA fragments... hybridization with labelled 58 *V. nigripulchritudo* genomes: 81 DNA fragments selected



Fonction of the 15 DNA fragments specifics of the vir+ strains of the cluster A

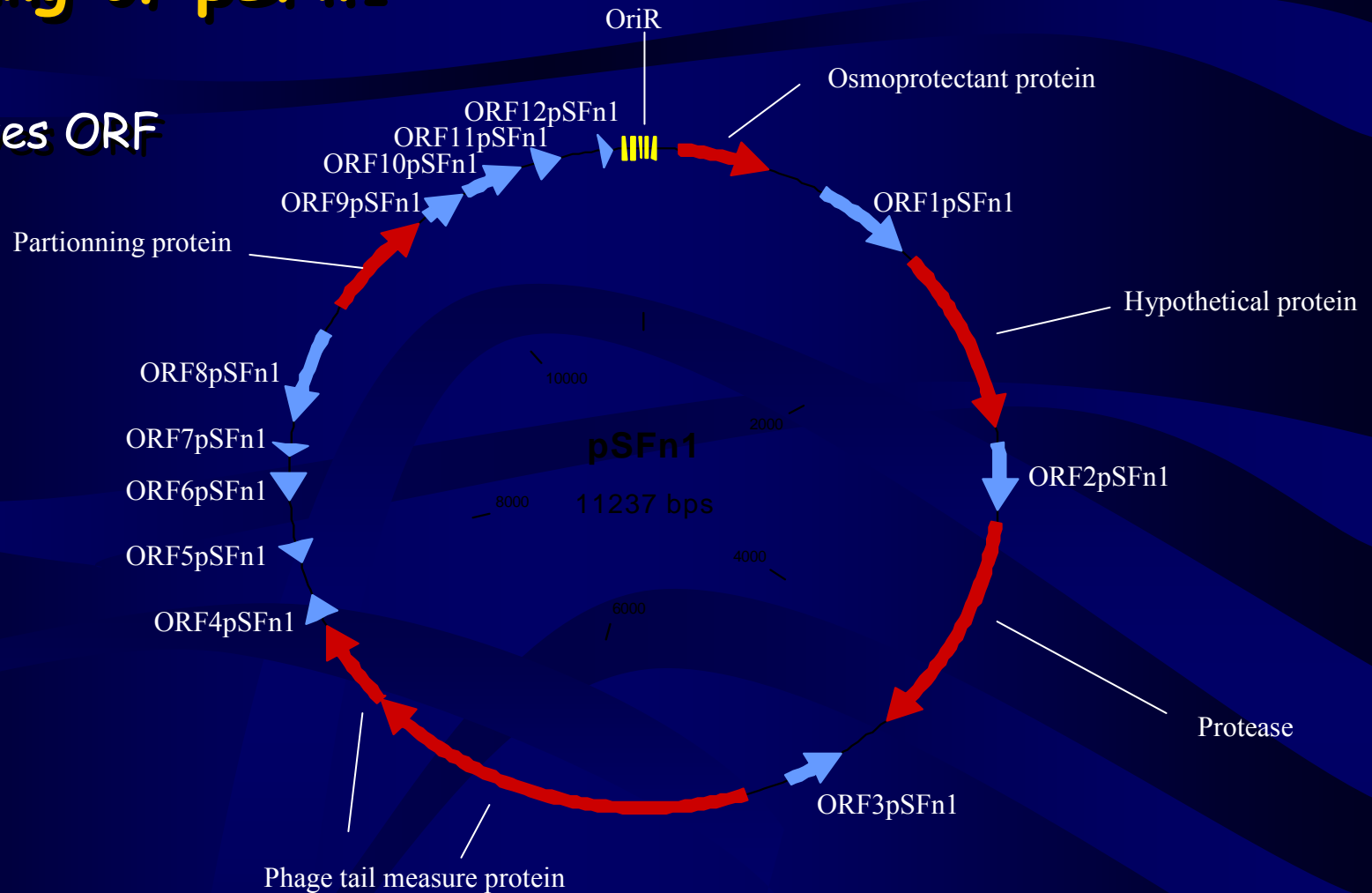
clone	blast
16	Vibrio shiloi 13.5 kb plasmid sequence Z2Z3
64	hypothetical protein VV0144 [Vibrio vulnificus YJ016]
68	Vibrio shiloi 13.5 kb plasmid sequence Z8
104	hypothetical protein Neut_2547 [Nitrosomonas eutropha C71]
147	Unknown COG
155	Vibrio shiloi 13.5 kb plasmid sequence Z8
191	predicted phage tail protein [Vibrio vulnificus]
225	Unknown COG
284	hypothetical protein R2601_22861 [Roseovarius sp. HTCC2601]
302	Phage tail tape measure protein TP901, core region [Thiomicrospira crunogena XCL-2]
378	Unknown COG
414	Unknown COG
506	Predicted transcriptional regulator [Vibrio alginolyticus 12G01]
522	putative tail length determinant [Bacteriophage K139]
535	Unknown COG

the 13,5kb plasmid of *Vibrio shilonii* associated with coral bleaching of *Oculina patagonica* (Kushmaro et al., 1996)

Looking for a plasmid in SFn1? (different technical approaches tested)

Sequencing of pSFn1

- 11237 bp
- 18 putatives ORF

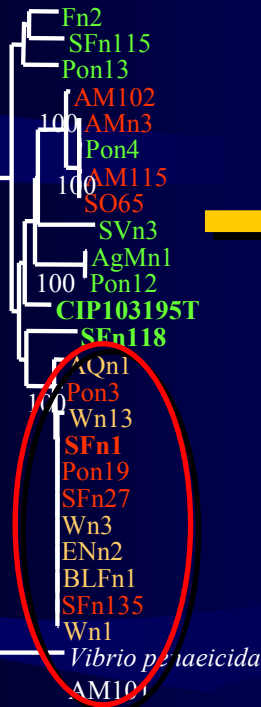


- 5 plasmidic DNA fragments of *V. shilonii* are present on pSFn1
- 12 of the 15 DNA fragments selected by SSH are plasmidic

Searching for plasmidic DNA in a collection of *V. nigripulchritudo* strains and prevalence of the Z2Z3 signal by southern blot

	strains	plasmid (s)	Z2Z3 signal in southern blot
vir+ cluster A	SFn1	+	+
	Pon19	+	
	SFn27	+	
	Pon3	+	
	SFn135	+	
vir+/- cluster A	AQn1	+	-
	BLFn1	-	
	ENn2	-	
	Wn1	-	
	Wn3	-	
	Wn13	-	
vir-	AgMn1	+	-
	SFn118	-	
	SFn115	-	
	Pon13	-	
	Pon4	-	
	SVn3	-	
vir+ cluster C	SO65	-	-
	AM115	-	
	AMn3	+	
	AM102	+	

Conclusion



• the correlation between taxonomic grouping and virulence suggests that a fraction of the virulence genes are chromosomal

• the plasmid is found only in the highly pathogenic strains associated to the summer syndrome

Hypothesis:

• The plasmid present one or more additional virulence genes

And/or

• the plasmid present a regulation factor of chromosomal virulence genes

• An horizontal transfer of plasmid between *V. shilonii* and *V. nigripulchritudo* could be suggested ...

Perspectives

- Transfert of **pSFn1** in **moderately virulent** and **non virulent** *V. nigripulchritudo* strains...test in experimental infection of the virulence potential
- Develop a mutagenesis approach in the plasmid or in chromosomes
- *Genome sequencing of the highly pathogenic Vibrio nigripulchritudo strain SFn1*



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Unity of Bacterial Genom Plasticity
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