

# Yellow Sticks Listens To Jazz

Small regulatory RNAs in *Xanthomonas campestris* pv. *vesicatoria*

Sven Findeiß

University Leipzig  
Department for Bioinformatics

Bled, February 2009

Yellow Sticks Listens To Jazzs

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# A Story about...

Yellow Sticks Listens  
To Jazz

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**Introduction**

Identification

Features of jasRNAs

Anti Sense RNAs

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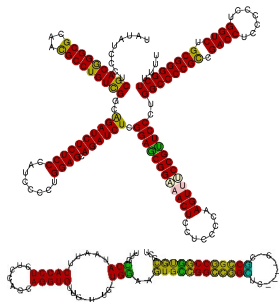


**Yellow Sticks,**

## Structured RNAs,



Yellow Sticks,



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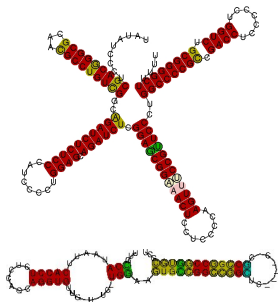
Features of jasRNAs

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## Structured RNAs,



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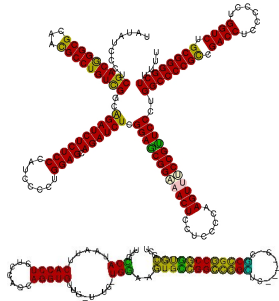


**and Black  
Spots.**

## Structured RNAs,



Yellow Sticks,



and Black  
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# Xcv – Causal Agent of Bacterial Spot Disease

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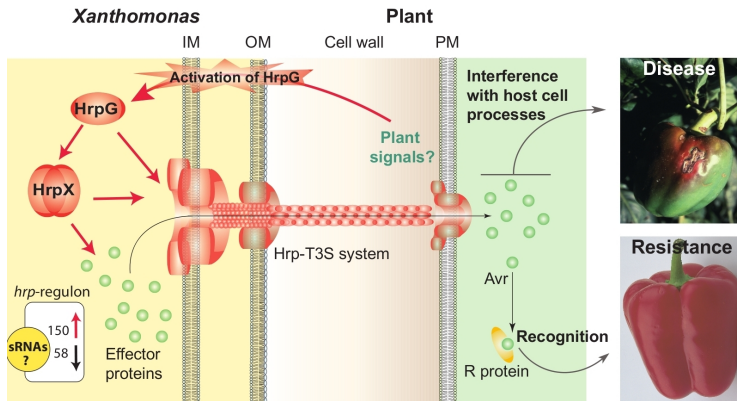
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- 85-10 ⇒ WT – strain
- HrpG\* – strain ⇒ 85-10 derivative with gene expression pattern like Xcv after plant contact



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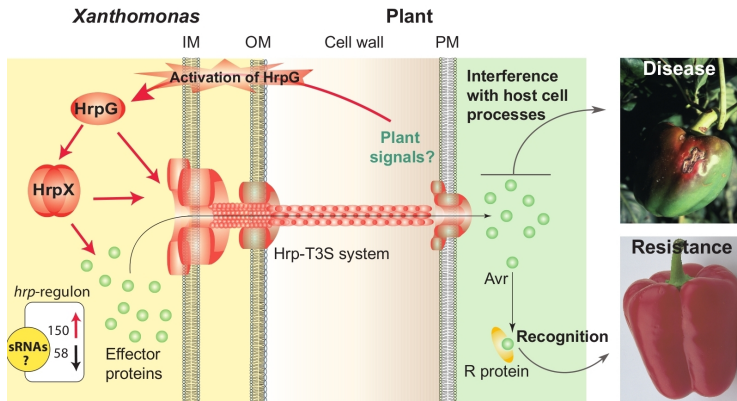
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**So far, nothing is known about small regulatory RNAs in the plant pathogen *Xcv*.**

???How to find these transcripts???

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# Transcriptome Sequencing (454)

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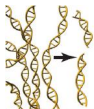
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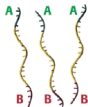
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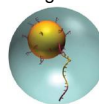
## 1) RNA Isolation



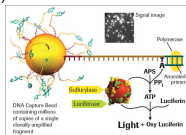
## 2) Fragment Preparation



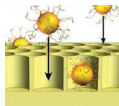
## 3) One Bead = One Fragment



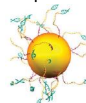
## 6) One Bead = One Read



## 5) PicoTiterPlate Fixation

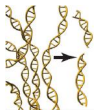


## 4) Amplification

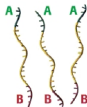


In cooperation with Cynthia Sharma and Joerg Vogel MPIIB Berlin

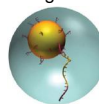
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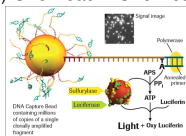
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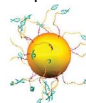


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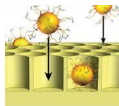


	Total Reads	Mapped Reads
1. Library	62,861	51,746
2. Library	51,450	42,015

## 4) Amplification



## 5) PicoTiterPlate Fixation

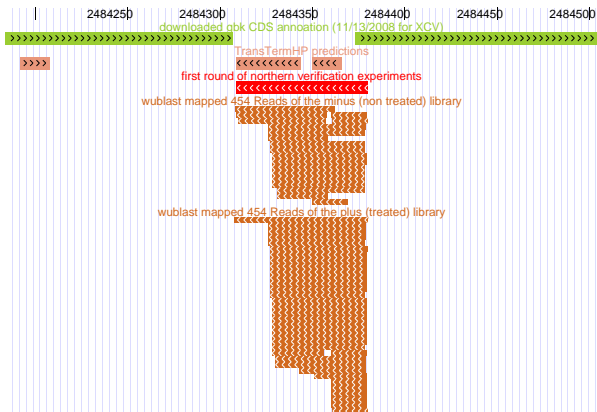


## Abundant (known) ncRNAs

	length nt	plus library #	minus library #
RNaseP_RNA	356	66	45
SRP_RNA	114	111	433
tmRNA	396	121	638
6S RNA	220	1444	497
<b>jasRNA</b>	<b>71</b>	<b>35</b>	<b>19</b>

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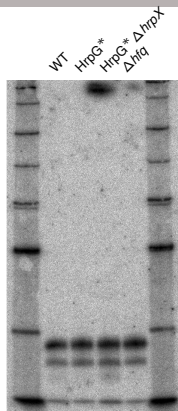
- abundant in 454 libraries
- ~70nt in length
- anti-sense to a protein coding gene



# The jasRNA is...

- abundant in 454 libraries
- ~70nt in length
- anti-sense to a protein coding gene
- constitutively expressed

⇒



Northern Blots: 20 $\mu$ g RNA, harvested in exponential growth stage; hybridization with 20nt oligonucleotides <sup>32</sup>P-labeled



# The XCV2162 Protein is...

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Using `tblastn`, homologs of the XCV2162 protein coding genes are searched. Each initial blast hit was extended to an complete open reading frame.



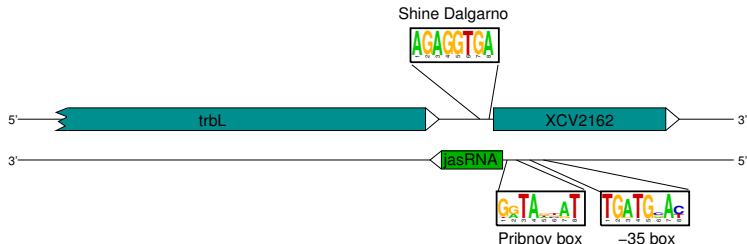
# The XCV2162 Protein is...

Using `tblastn`, homologs of the XCV2162 protein coding genes are searched. Each initial blast hit was extended to an complete open reading frame.



- conserved in various species
- 75-97 amino acids coding gene
- totally conserved (\*) and by substitutions (':' and '.') supported alignment columns

The upstream region (100nt) of the jasRNA and XCV2162 were analyzed using MEME.



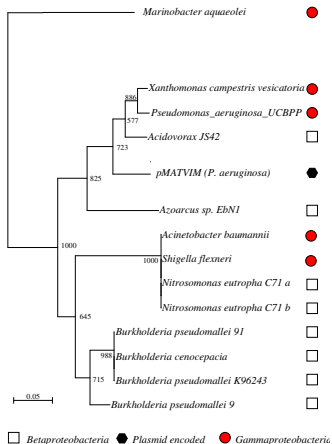
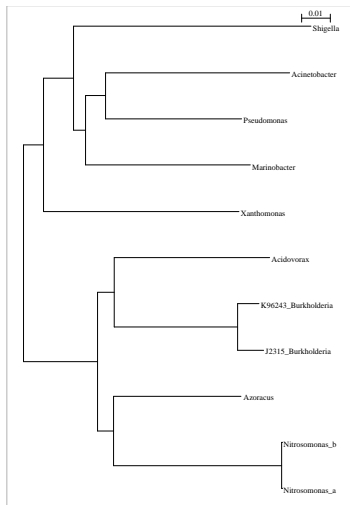
## jasRNA Promoter:

- two motifs found:
  - 13-12nt upstream → Pribnov box (-10 box)
  - 42-36nt upstream → -35 box
- subsequent PRODORIC database search revealed a 'Sig70 (-10)' pattern from position 3-8 of the first MEME motif

## XCV2162 Promoter:

- \* AG rich motif found directly upstream of the XCV2162 → Shine Dalgarno

# Phylogenetic distribution



## Functions of known anti-sense Systems

### 1 **Type I toxin-antitoxin:**

High levels of the toxin lead rapidly to cell death (as was observed by decreased optical density and colony forming ability).

### 2 **Others:**

Mechanisms as for instance SOS response and growth regulation are known to be regulated by anti-sense systems.



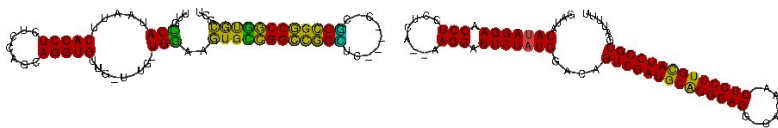
TABLE 1. Characteristics of type I toxin-antitoxin systems

System	Plasmid or bacterium	Discovery	Protein length (amino acids)	Protein expression	RNA length (nucleotides)	RNA expression	Gene arrangement <sup>a</sup>	Reference(s)
Hok-Sok	R1/R100/F	Plasmid stability	50	Upon plasmid loss	67	Constitutive		18, 22, 34, 37, 43
SrnB-SrnC	F	RNA degradation	49	Upon plasmid loss	63	Constitutive		20, 29, 36, 37, 39
PndA-PndB	R483	RNA degradation	50	Upon plasmid loss	66	Constitutive		2, 20, 29, 36, 38
RNAI (Fst)-RNAII	pAD1	Plasmid stability	33	Upon plasmid loss	66	Constitutive		24, 54, 55
Hok-Sok	<i>E. coli</i>	Homologs of plasmid-encoded system	-50	Not known	-65	Not known		45
Ldr-Rdl	<i>E. coli</i>	Identified as repeats	35	Not known	60	Constitutive		31
Ibs-Sib	<i>E. coli</i>	Identified as repeats	18-19	Not known	-110, -140	Constitutive		12
TisB-IstR-1	<i>E. coli</i>	Computational search for sRNAs	29	Induced by SOS	75	Constitutive		53
ShoB-OshC	<i>E. coli</i>	Cloning-based search for sRNAs	26	Not known (mRNA is highest in exponential phase)	-60	Highest in stationary phase in minimal medium		12, 32
SymE-SymR	<i>E. coli</i>	Cloning-based search for sRNAs	113	Induced by SOS	77	Constitutive		30, 32
TpxA-RatA	<i>B. subtilis</i>	Array-based search for sRNAs	59	Throughout growth	222 + shorter products	Throughout growth		49

<sup>a</sup> The mRNA is shown as a thin arrow and the sRNA as a thick arrow. The toxin open reading frame is indicated by a box. The sizes are approximate.

## jasRNA-XCV2162 hallmarks

- 79nt in length
- anti-sense to *traJ* 5'UTR
- 9nt overlap with *traJ* ORF
- similar in structure and conservation to the jasRNA



jasRNA

FinP

- so far annotated on *E. coli*, *Salmonella* and *Klebsiella* plasmids

## FinP One of the Others

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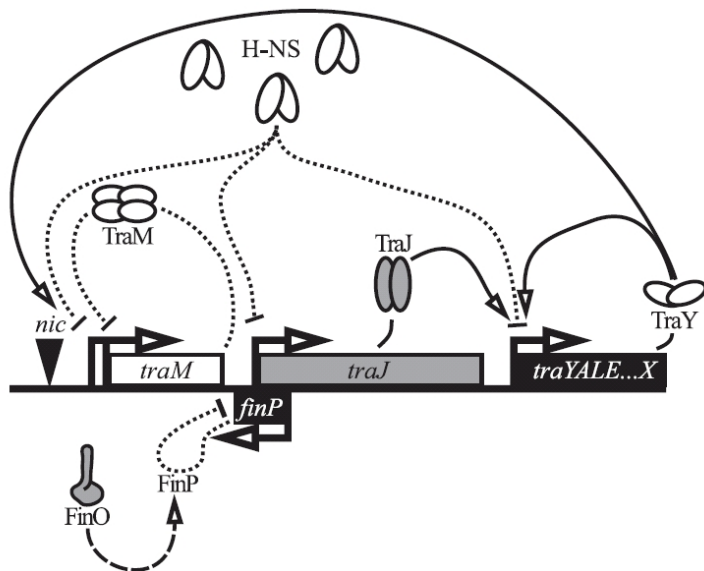
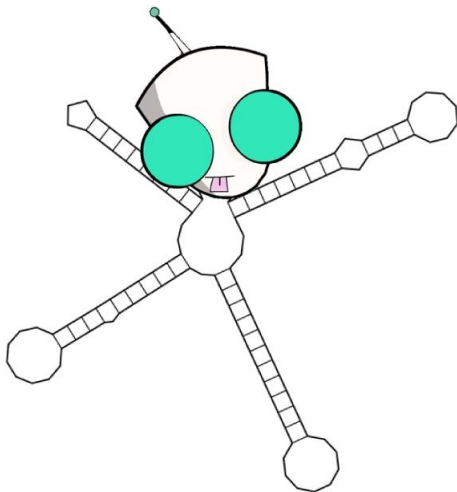
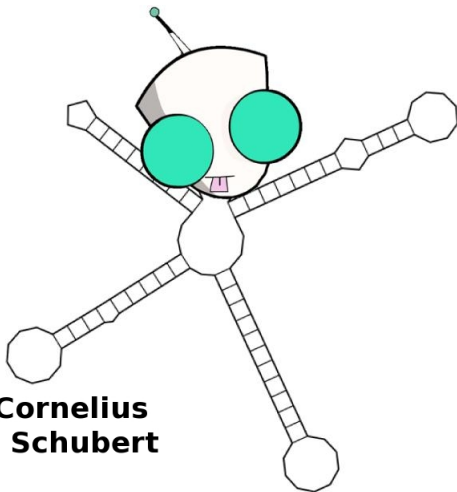


Figure taken from: Will et al, *Hfq Is a Regulator of F-Plasmid TraJ and TraM Synthesis in Escherichia coli*, 2006, Journal of Bacteriology





**Cornelius  
Schubert**

