Small regulatory RNAs in Xanthomonas campestris pv. vesicatoria

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University Leipzig Department for Bioinformatics

Bled, February 2009

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Introduction

Identification

Features of jasRNAs

Small regulatory RNAs in Xanthomonas campestris pv. vesicatoria

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Yellow Sticks Listens To Jazzs

Yellow Sticks Listens To Jazz

Sven Findeiß

Introduction

Identification

Features of jasRNAs

A Story about...

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Sven Findeiß

Introduction

Identification

Features of jasRNAs

Sven Findeiß

Introduction

Identification

Features of jasRNAs

Anti Sense RNAs



Yellow Sticks,

A Story about...



Yellow Sticks,

Structured RNAs,



Yellow Sticks Listens To Jazz

Sven Findeiß

Introduction

dentification

eatures of jasRNAs

A Story about...



Yellow Sticks,

Structured RNAs,





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Sven Findeiß

Introduction Identification Features of jasRNAs

and Black Spots.



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Introduction Identification Features of jasRNAs Anti Sense RNAs

Xcv – Causal Agent of Bacterial Spot Disease



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Sven Findeiß

Introduction

dentification

eatures of jasRNAs

Anti Sense RNAs

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

Thieme et al. J Bacteriol, 2005; Koebnik et al. J Bacteriol 2006

Xcv – Causal Agent of Bacterial Spot Disease





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Introduction

dentification

eatures of jasRNAs

Anti Sense RNAs

• 85-10

 \Rightarrow WT – strain

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

Sven Findeiß

Introduction

Identification

Features of jasRNAs

Anti Sense RNAs

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

???How to find these transcripts???

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Introduction

Identification

Features of jasRNAs

Anti Sense RNAs

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

???How to find these transcripts???

Trascriptome Sequencing (454)









4) Amplification

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Introduction

Identification

eatures of jasRNAs

Anti Sense RNAs





5) PicoTiterPlate Fixation

In cooperation with Cynthia Sharma and Joerg Vogel MPIIB Berlin

Trascriptome Sequencing (454)





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Sven Findeiß

Introduction

3) One Bead = One Fragment

4) Amplification

Identification

eatures of jasRNAs

Anti Sense RNAs

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

6) One Bead = One Read



5) PicoTiterPlate Fixation



In cooperation with Cynthia Sharma and Joerg Vogel MPIIB Berlin

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
jasRNA	71	35	19

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Sven Findeiß

Introduction

Identification

eatures of jasRNAs

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Sven Findeiß

Introduction

Identification

Features of jasRNAs

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

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Introduction

Identification

Features of jasRNAs

The jasRNA is...

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Introduction

Identificatior

Features of jasRNAs

Anti Sense RNAs



- \sim 70nt in length
- anti-sense to a protein coding gene
- constitutively expressed



 \Rightarrow

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

The jasRNA is...

W HIP HODNA

- abundant in 454 libraries
- \sim 70nt in length

Burkho Burkho Burkho Burkho Pseudo Adidow Pseudo Xantho Azoaro Marino Nitros Shigel

- anti-sense to a protein coding gene
- constitutively expressed
- structurally conserved with various compensatory mutations

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Sven Findeiß

Introduction

Identificatior

Features of jasRNAs

Anti Sense RNAs

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2		

			30
	[[[]]	
olderia_denodepadia_J2315	UUG	UACUUCA	CUCAA
olderia_pseudomallei_K96243	UUGC	UACUU	CUCAA
deria_pseudomallei_9	TUGC	UGCUU A	CUCUA
olderia_pseudomallei_91	UUGC	UACUUCA	CUCAA
monas-acruginosa-phATVIN-7	UUGC	NUGAUU A	CUGUG
OTAX_JS42	UUGC	UGAUU	CUCUA
monas_aeruginosa_UCBPP-PA14	TUG	UGAUUCA	CUCCA
monas_dampestris_vesidatoria_85-10	TUG	UGAUUCAC	CUCCA
ius_sp_EbN1	UUGC	UGAUU	CUCCA
badter_aquaeolei_VTB	CUGU	UGUGU	CUCUG
comonas_eutropha_CS1_a	UUGU	UAAUU	CUCCA
comonas_eutropha_C91_b	UUGU	AUAAUUCAU	CUCCA
la_flexneri_2a_2457T	UUGU	UAAUU	CUCCA
obadter_baumannii_ATCC_17978	UUGU	UAAUU	CUCCA
	1000		and the owner of the
		and the second s	

 \Rightarrow

stage; hybridization with 20nt oligonucleotides 32 P-labeled									
[]	··· [[[····· [[[[[······]]]]]······]]]·· [[[[[[[[
UUG	UACUU	CICAAGC	UUGUUG-U	GAAGUGCC	CCG	UGC-C	CGG	GGUGC	GCU
UUG	UACUU	CUCAAGC	UUUGUUG-U	GAAGUGGC	CCG	UGC-C	CGG	Genec	GCU
UUG	UGCUU	CUCUAGO	UUGAUACG-	GUGGC	COC C		eeee	GGUGC	GCU
THE	UNCOUL	CHCHCGC		CHGUGUC	CCC C		200	COCCO	CCT
TILG	UGAUU	CIICIDAC	GGII-IIG-	AAGIICIC	1000	CCCAC	acce.	Gacac	GCII
UUG	IGAUU	CUCCAUCAGE	GGU-UAA	AAGUGUC	CCG	CCCAC	CGG	GACGO	GCC
UUG	UGAUU	COLCUCCAUCAGE	GGU-UAG	GAAGUGUC	CCGC	CCUAC	CGGC	GACGO	GCC
UUGC	UGAUU	CUCCAUCAG	UGUU-GAG	GACGUGCC	CUGO	CCGCAAU	CGGC	GGCAC	GCU
CUG	UGUGU	CUCUGUCAGE	GUA-UG-U	GAAAUGCG	CCGE	GCACCAU-C	CGGC	CGUGE	GCU
UUG	UAAUU	CUCCAGU	UUUGAU	RAAGUGCC	GOUL	GUUGUGG	AGC	GOUGO	GCC
UUGU	UAAUU	CUCCAGU	UUGAU	GUGGGC	GCU	UUGUGG	AGC	GGUGC	GCC
UUG	UAAUU	CUCCAGU	UUGAU	CHOCK			AGC	GOUGO	GCC
uudu	UNAUU	Cucchou	u u u u u u u	AN BOOK	SC U	000000	and a	aguad	occ
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Northern Blots: 20µg RNA, harvested in exponential growth

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

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Introduction

Identification

Features of jasRNAs

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.

	Rec : : : : : : : : : : : : : : : : : : :
Burkhalderia	MATMED TE ARE VI HETAMRIAAIE PID RHAAKD ISOMAEAVANEP VVVP YOAE TGRATESD PREAMTAVEOAIOOYAE PPKRYD VI SGEGANKVWS-
Burkhalderia	MATMHDTE ARE VI HHTAMOLAALEP IDPHAAKDLSEMTEAMVNLF VVVF YOAETGRATRSDPREAMTAVROALLOYAEOLKCHGGI SGKGANEAWS-
Burkhalderia	MATTHNTE ACOVI HHAATOLAD LDPMOORTARE LSKMAEAVAN LPMVVP YOAE TGRATELDP SEAMAAVEE LORH
Burkhalderia	MATMEDTE ARE VI HETAMOLAALEPIDPHAARDLSEMTEAMVNLPVVVPYOAETGRATESDPREAMTAVROALLOYAEOLECHGGISGEGANEAWS-
Panudorunaa	MATLNPTNALATOAVHHAAAQLAALDWIDQEAARQLSPMAEAVANMPMVLYYQAETGQATRDDFRQALDAVRQSLTA
Anidovozaz	MATLNPTN-ATQAVHHAAVQLAALDWLDQDAARQLGPLAEAVANAPMVVFYQAETGQATFADFREALDAVRQSLGAA
Paradoromaa	MATLNETN-ATQAVHHAAVQLAALDWIDQDAARQLGELAEAVANAFMVVFYQAETGRATEADFREALNAVRQSLHEA
Lasthornas	MATLNETN-ATQAVHRAAVQLAALDWIDQDAARQLGELAEAVANAFMVVFYQAETGRATEADFREALNAVRQSLHEA
Asparous	MATRHATE ATE IVE QAAARITAIE WODGE GAROI SPILAEAVANMY VVLFY QAE TO QATFADFHEAIAAVROTLAT
Marianhantar	MTATHNIE VOQAIRQAATKLSALKFIDQETARQISPVAEAVANMFTILYYQAETGRATQEDFQEALATIRQATEYDER
Wit.roscence.	MTTORI EPGOAVROAAAILSSLEYINQAEARSLGPLAEAVANAPMVVYYQAETGRATOADFOEAMNALROACS
Mit.roscence.	MTTCHIIEPGQAVHCAAAILSSLEYINGAEARSLGPLAEAVANAPMVVYYGAETGRATGADFOEAMNALROACS
Shigalla	MTTCHIIEPGQAVHQAAAILSSLEYINQAEARSLGPLAEAVANAPMVVYYQAETGRATQADFOEAMNALROACS
Aninetohester	MTTOHILEPGOAVHOAAAILSSLEYINOAEARSLGPLAEAVANAPMVVYYOAETGRATOADFOEAMNALROACS
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Introduction

Identification

Features of jasRNAs

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

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Introduction

Identification

Features of jasRNAs

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



jasRNA Promoter:

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

XCV2162 Promoter:

 $\ast\,$ AG rich motif found directly upstream of the XCV2162 \rightarrow Shine Dalgarno

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Introduction

dentificatior

Features of jasRNAs

Phylogenetic distribution



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Introduction

dentification

Features of jasRNAs

Functions of known anti-sense Systems

• 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).

Others:

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

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Introduction

Identification

Features of jasRNAs

Anti Sense Systems

Downloaded from mmbr.asm.org at Vienna University Library on February 1, 2009

	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*	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	E. coli	Homologs of plasmid- encoded system	-50	Not known	-65	Not known	← ■→	45	
Ldr-Rdl	E. coli	Identified as repeats	35	Not known	60	Constitutive	←	31	
Ibs-Sib	E. coli	Identified as repeats	18-19	Not known	-110, -140	Constitutive	→	12	
TisB-IstR-1	E. coli	Computational search for sRNAs	29	Induced by SOS	75	Constitutive	←■>	53	
ShoB-OhsC	E. coli	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	E. coli	Cloning-based search for sRNAs	113	Induced by SOS	77	Constitutive	÷ martin →	30, 32	
TpxA-RatA	B. subtilis	Array-based search for sRNAs	59	Throughout growth	222 + shorter products	Throughout growth		49	

"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.

Table taken from: Fozo et al, Small Toxic Proteins and the Antisense RNAs That Respress Them, 2008, Microbiology and Molecular Biology

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Introduction

Identification

Features of jasRNAs

FinP One of the Others

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



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

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Introduction

Identification

Features of jasRNAs

FinP One of the Others



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

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Introduction

dentification

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711

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eatures of jasRNAs



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Introduction

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Features of jasRNAs



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Introduction

Identification

eatures of jasRNAs