Post-transcripcional regulation of genes involved in polyhydroxybutyrate synthesis in *Azotobacter vinelandii*

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FAPESP
Workshop for Sustainable Production of Biopolymers and other Bio-based Products
July 25th - FAPESP - São Paulo

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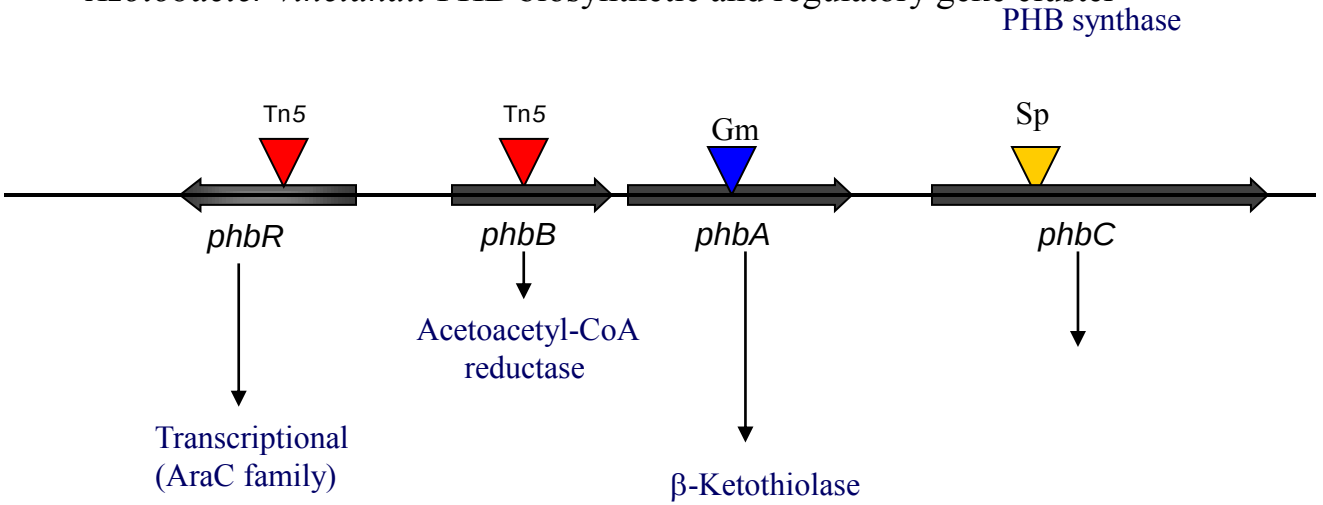
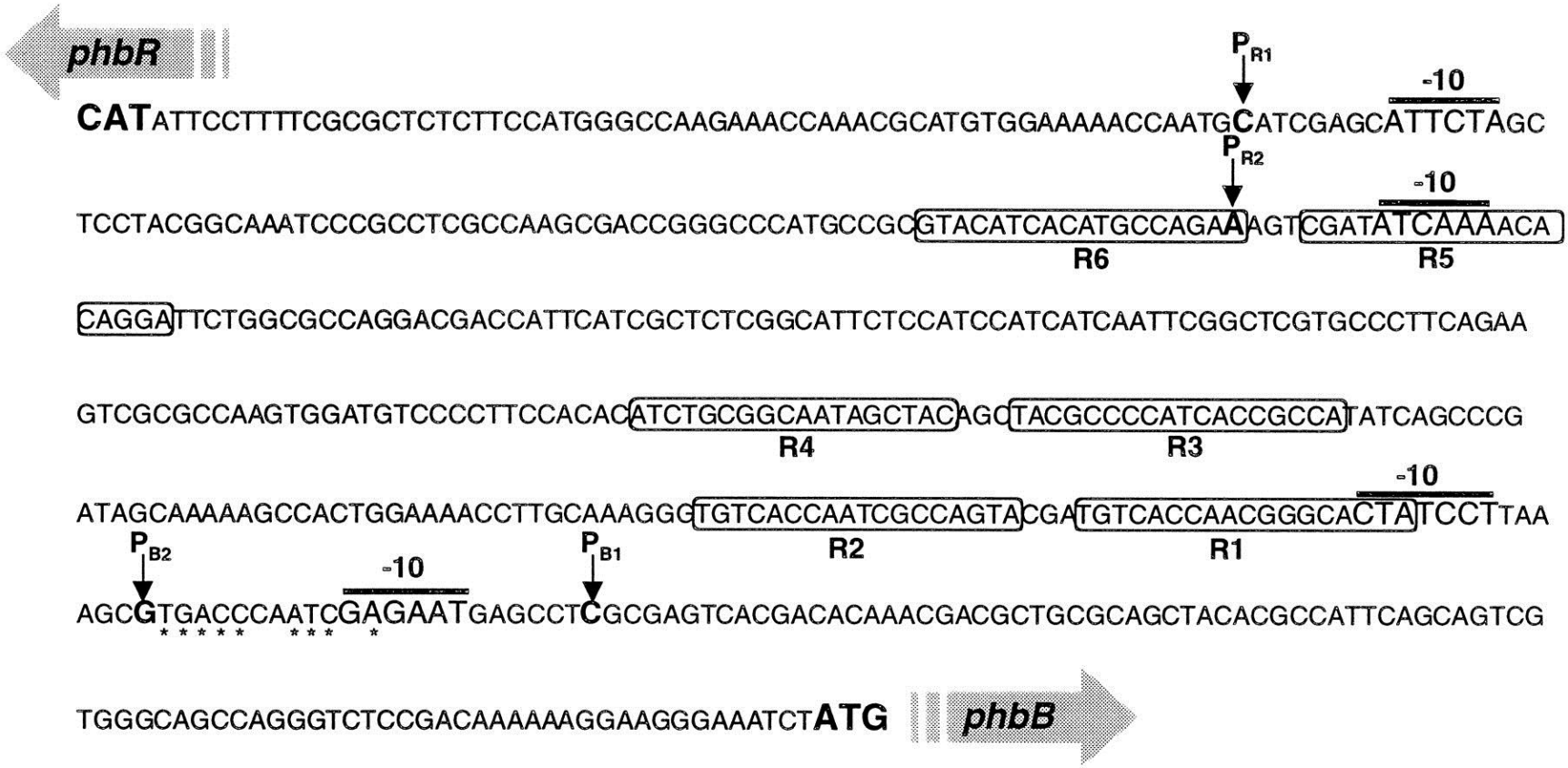
July 2012

Azotobacter vinelandii PHB biosynthetic and regulatory gene cluster



Segura et al 2003, Arch Microbiol 179:437-443



Intergenic *phbR-phbB* region. The arrows indicate transcription start sites. The phbB and phbR start codons are indicated by boldface type. The -10 sequences of pB1, pB2, pR1, and pR2 are overlined. Sites R1 to R6 are enclosed in boxes.

(Peralta-Gil 2002 J. Bacteriol 184:5672-5677)

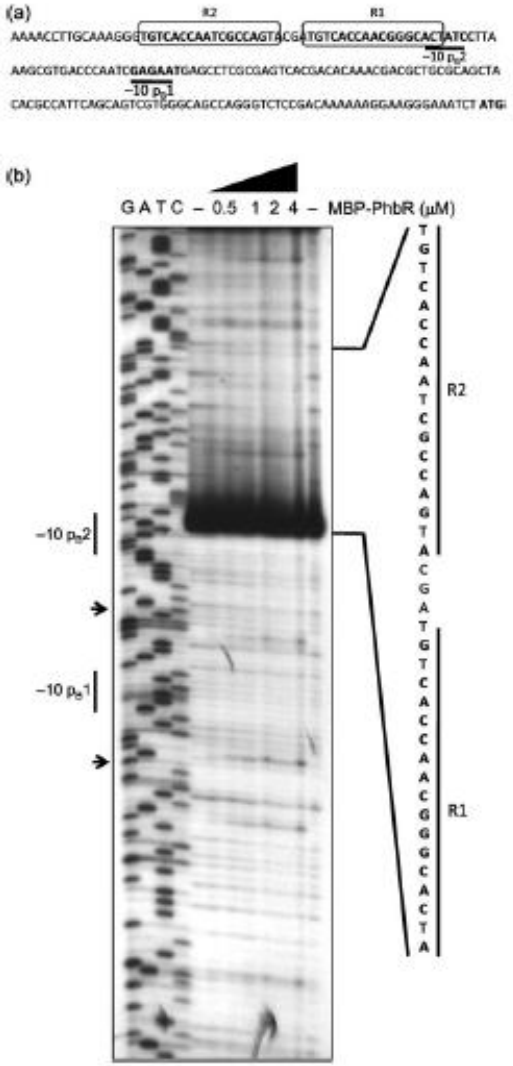
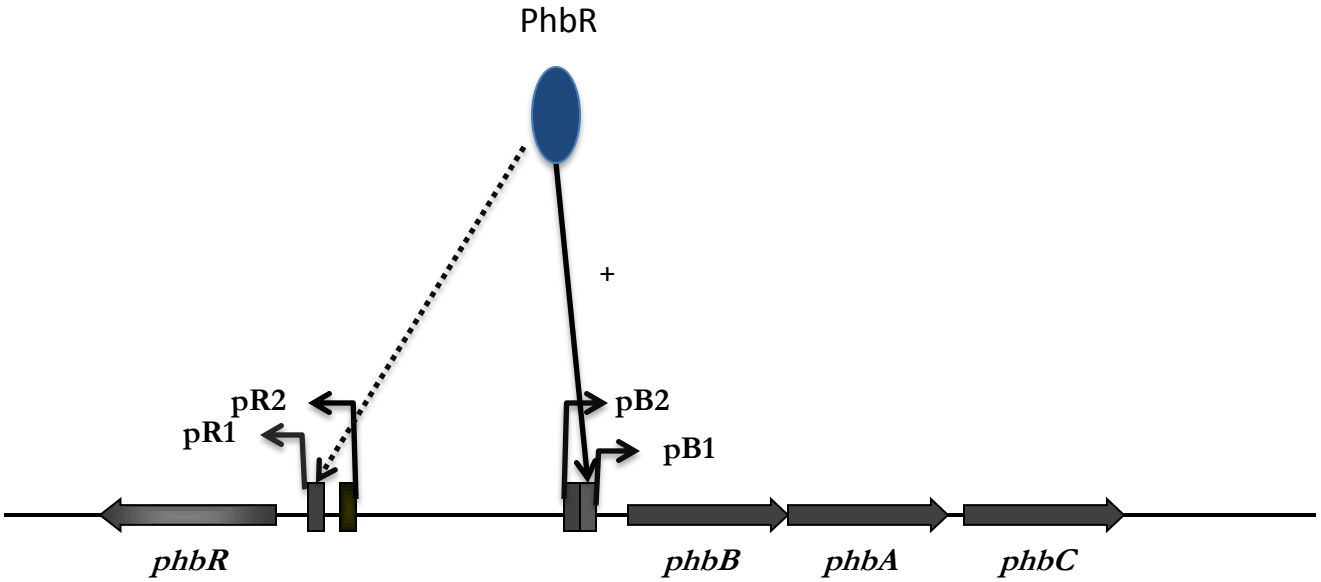
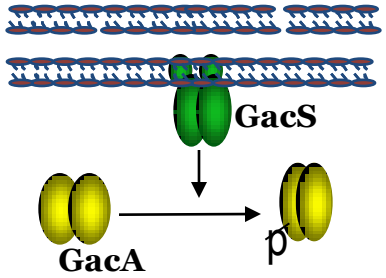


Fig. 6. (a) The *phbB* promoter region. The R1 and R2 sequences are boxed. Asterisks indicate the transcription start sites reported by Peralta-Gil *et al.* (2002). The −10 regions for p_B1 and p_B2 are underlined. (b) DNase I footprinting analysis of the interaction of PhbR with the *phbB* promoter region. The reaction mixture was treated as described in Methods, and the sequencing ladder was generated with plasmid pCT2P and oligonucleotide footphbR4. –, Absence of PhbR. The −10 regions of the p_B1 and p_B2 promoters are indicated. The arrows show the start sites for *phbB* transcription. The R1 and R2 sequences protected from degradation are also shown.

PhbR is a transcriptional activator of the *phbBAC* operon in *Azotobacter vinelandii*



The GacS-GacA two component system also controls the synthesis of PHB in *A. vinelandii*



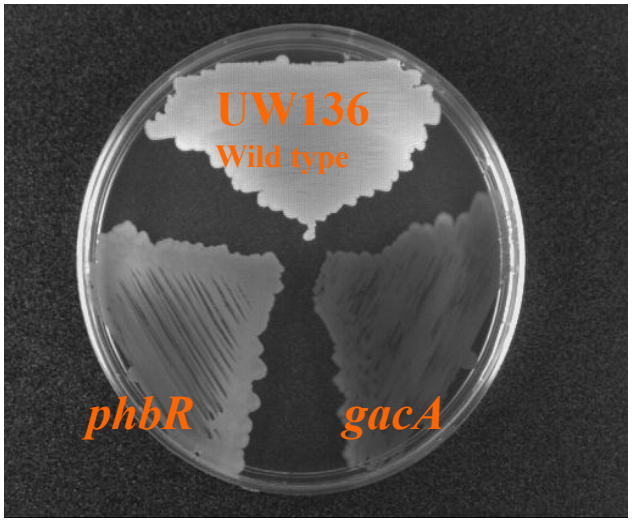
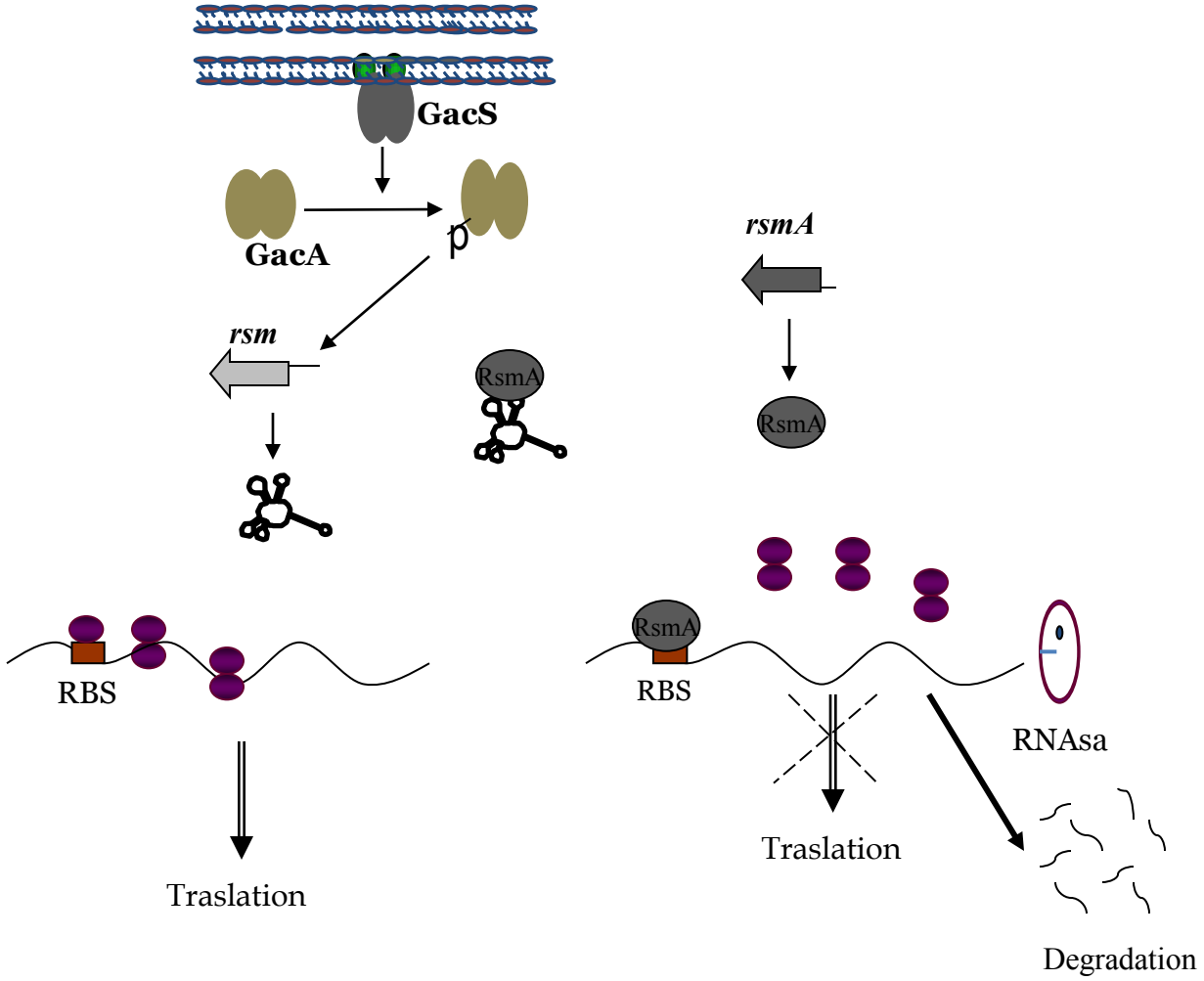


Table 1. Mutants affected in *gacS* and *gacA* homologues in γ-proteobacteria.

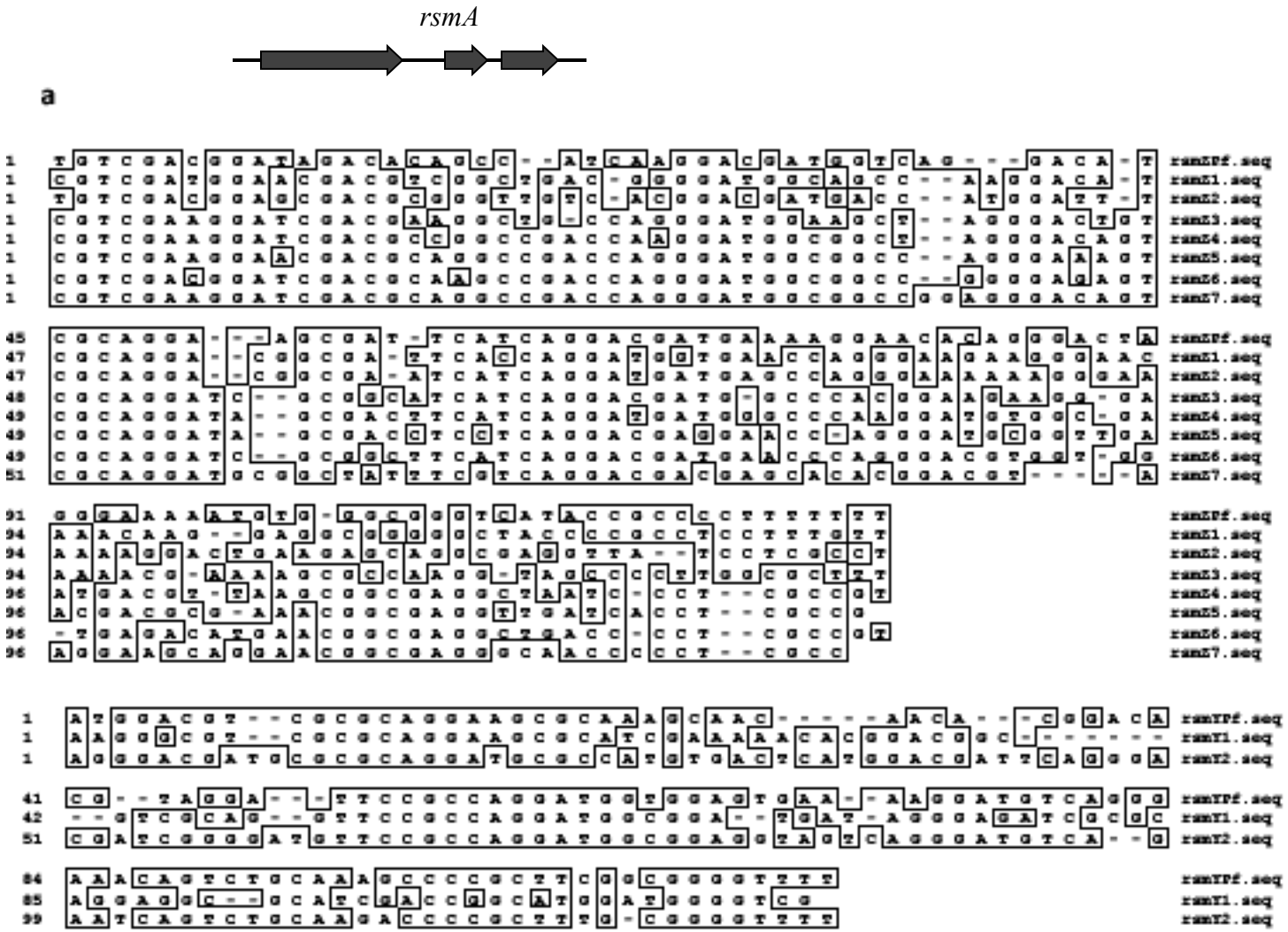
Species	GacS/GacA homologues	Major GacS/GacA-controlled phenotypes	GacS/GacA-dependent sRNAs	References
Acinetobacter baumannii	GacS/GacA	Citrate utilization	RsmX, RsmY, RsmZ[a]	Dorsey *et al.* (2002)
Azotobacter vinelandii	GacS/GacA	Alginate, poly-β-hydroxy-butyrate, encystment	?	Castañeda *et al.* (2001)
Erwinia carotovora ssp. *carotovora*	GacS/GacA (ExpS/ExpA)	Extracellular pectinases, cellulase, protease, virulence, motility	RsmB	Cui *et al.* (2001)
Erwinia chrysanthemi	?/GacA	Extracellular pectinases, cellulase, protease, TTSS, virulence	?	Lebeau *et al.* (2008)
Escherichia coli	BarA/UvrY	Central carbon metabolism, biofilm, virulence[b], motility	CsrB, CsrC	Suzuki *et al.* (2002); Weilbacher *et al.* (2003); Tomenius *et al.* (2006)
Legionella pneumophila	LetS/LetA	Cytotoxicity, virulence, motility	RsmY, RsmZ[a]	Hammer *et al.* (2002); Molofsky and Swanson (2003)
Pseudomonas aeruginosa	GacS/GacA	AHL, HCN, pyocyanin, lipase, elastase, biofilm, virulence, motility	RsmY, RsmZ	Rahme *et al.* (1995); Reimmann *et al.* (1997); Parkins *et al.* (2001); Kay *et al.* (2006)
Pseudomonas chlororaphis (*aureofaciens*)	GacS/GacA	AHL, phenazines, HCN, surfactants, 2,3-butanediol, protease, biocontrol	?	Chancey *et al.* (1999); Schmidt-Eisenlohr *et al.* (2003); Han *et al.* (2006); Girard *et al.* (2006)
Pseudomonas entomophila	GacS/GacA	Protease, hemolysin, virulence	RsmY, RsmZ[c]	Vodovar *et al.* (2006)
Pseudomonas fluorescens	GacS/GacA	DAPG, HCN, pyoluteorin, pyrrolnitrin, protease, phospholipase, biocontrol, H_2O_2 resistance, motility	RsmX, RsmY, RsmZ	Haas and Défago (2005); Kay *et al.* (2005); Heeb *et al.* (2005); Dubuis *et al.* (2007)
Pseudomonas marginalis	LemA/GacA	Pectinases, virulence	?	Liao *et al.* (1997)
Pseudomonas syringae pv. *syringae*	GacS(LemA)/ GacA	Syringomycin, syringolin, AHL, alginate, protease, virulence	RsmX, RsmY, RsmZ[c]	Willis *et al.* (2001); Quinones *et al.* (2004)
Pseudomonas syringae pv. *tomato*	GacS/GacA	Coronatine, AHL, TTSS, virulence	RsmX[a], RsmY, RsmZ	Chatterjee *et al.* (2003)
Pseudomonas tolaasii	PheN(RtpA)/?	Tolaasin, protease, virulence, motility	?	Grewal *et al.* (1995); Murata *et al.* (1998)
Pseudomonas viridiflava	RepA/RepB	Extracellular pectinase, protease, alginate, virulence	?	Liao *et al.* (1996)
Salmonella enterica ssp. Typhimurium	BarA/SirA	TTSS, invasion, motility	CsrB, CsrC	Altier *et al.* (2000); Fortune *et al.* (2006)
Serratia marcescens	PigW/PigQ	Prodigiosin	?	Williamson *et al.* (2006)
Serratia plymuthica	GrrS/GrrA	Extracellular protease, pyrrolnitrin, biocontrol	?	Ovadis *et al.* (2004)
Vibrio cholerae	VarS/VarA	HapR-dependent virulence factors	CsrB1[a], CsrB2[a] (= CsrC), CsrB3[a] (= CsrD)	Lenz *et al.* (2005)
Vibrio fischeri	GacS/GacA	Bioluminescence, squid colonization	CsrB1[a], CsrB2[a]	Whistler and Ruby (2003)

a. Predicted by Kulkarni *et al.* (2006).
b. In uropathogenic strains.
c. Predicted by BLASTN.
AHL, *N*-acyl-homoserine lactone; DAPG, 2,4-diacetylphloroglucinol; TTSS, type III secretion system; ? indicates that GacS/GacA-dependent sRNAs or regulators have not yet been identified.

Lapouge el at 2008. Mol. Microbiol 67:241-253



The two component GacS-GacA system regulates PHB synthesis throughout the Rsm system?



An *rsmA*, 7 *rsmZ* and one *rsmY* genes are present in the *A. vinelandii* genome

Manzo et al 2011, J. Mol. Microbiol. Biotechnol 21:147-159

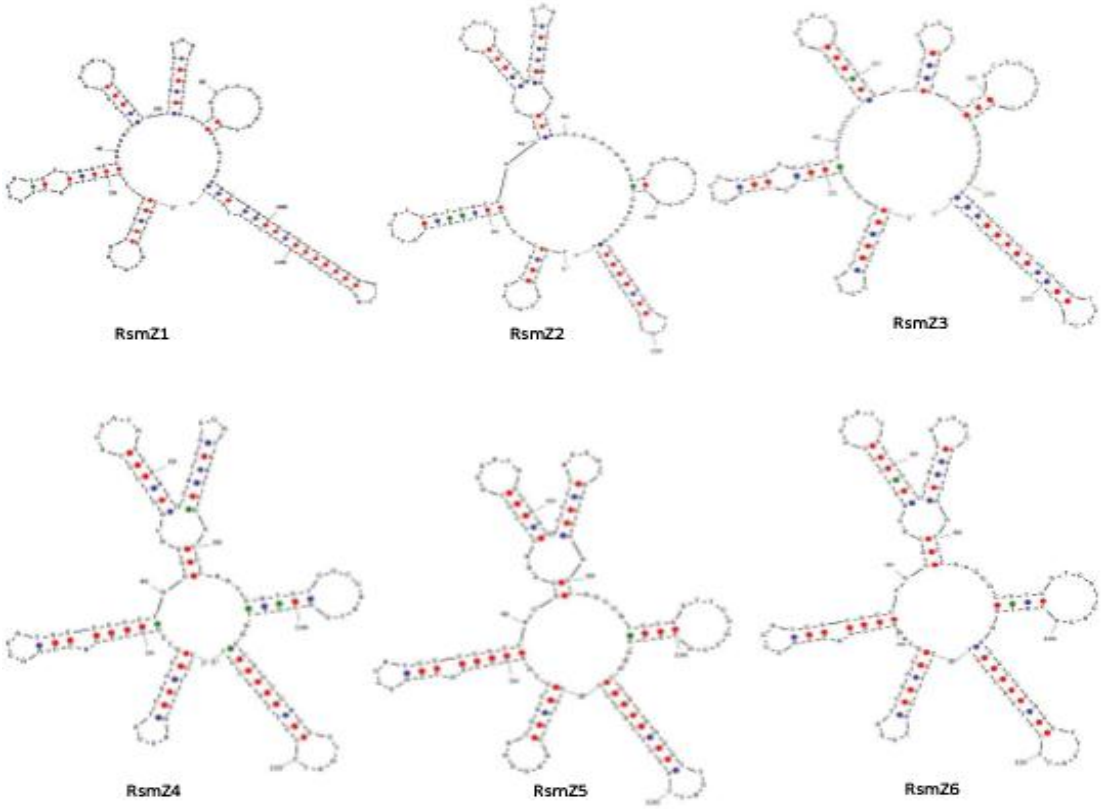


Fig. 2

Manzo et al 2011, J. Mol. Microbiol. Biotechnol 21:147’159

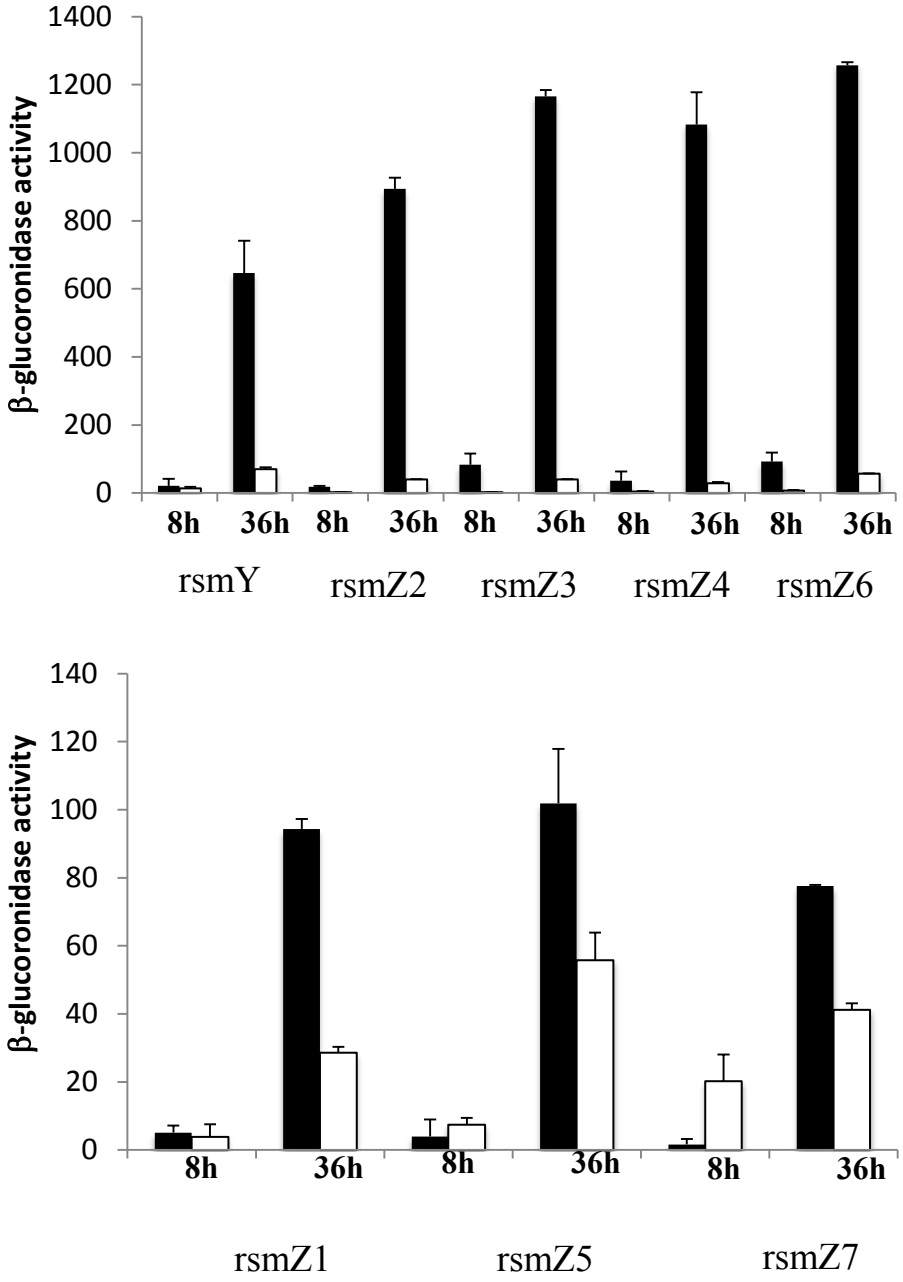


Fig. 1. GacA activates the expression of rsm regulatory RNAs. a) b-glucuronidase activity of rsmY and rsmZ-*gusA* gene fusion in the wild type UW136 (white bars) and in the isogenic *gacA* mutant AHgacA (black bars).

Hernandez-Eligio et al 2012, Microbiology 158: in press

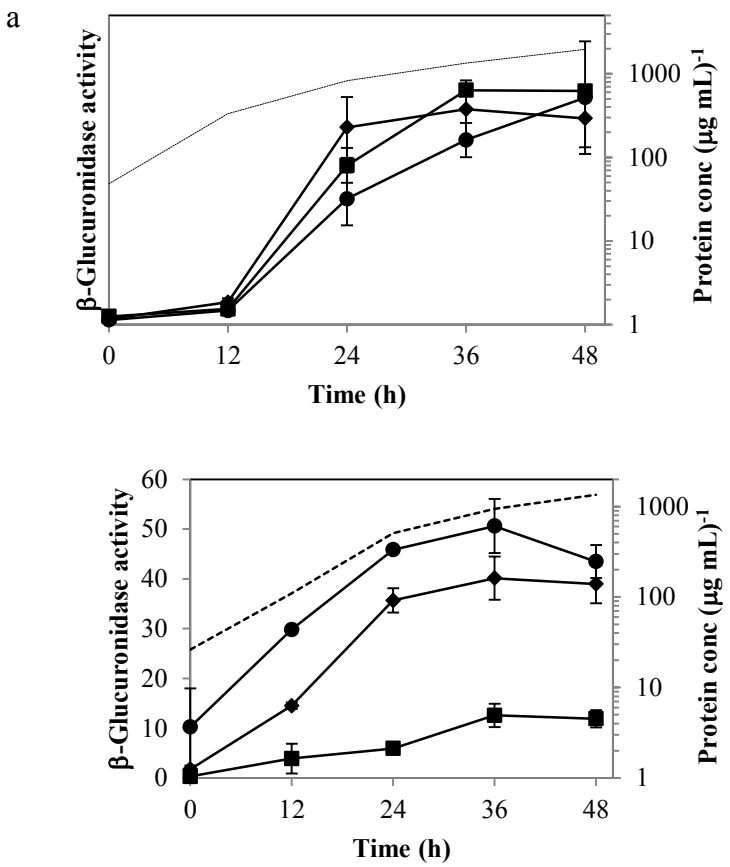


Fig. 2. GacA controls expression of *phbR* at the post-transcriptional level. β-glucuronidase activity in wild type (diamonds), *rsmA* (circles) and *gacA* (squares) strains. The dashed line in all graphs represents growth curves. a) β-glucuronidase activity of strains carrying a *phbR-gusA* gene transcriptional gene fusion or b) *phbR-gusA* translational gene fusion.

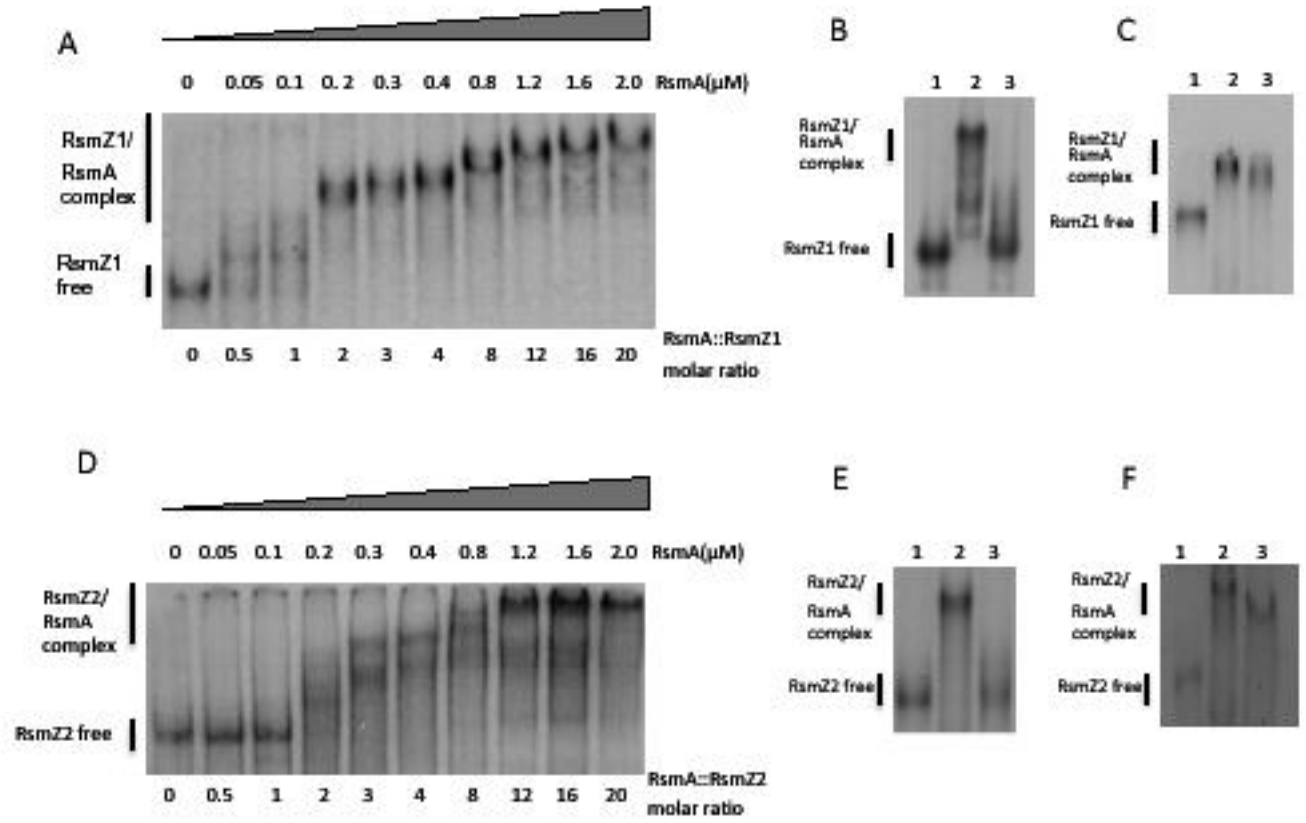
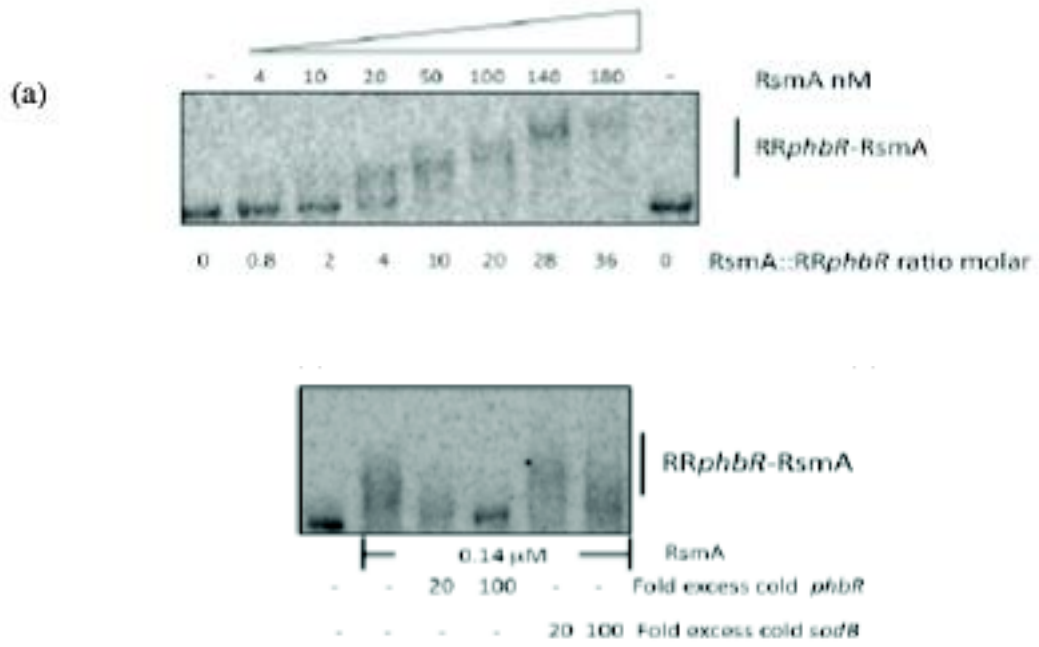


Fig. 4.

In vitro interacion of the RsmA protein with RsmZ1 and RsmZ2 RNAs

Manzo et al 2011, J. Mol. Microbiol. Biotechnol 21:147-159



In vitro interaction of RsmA with the *phbR* transcript

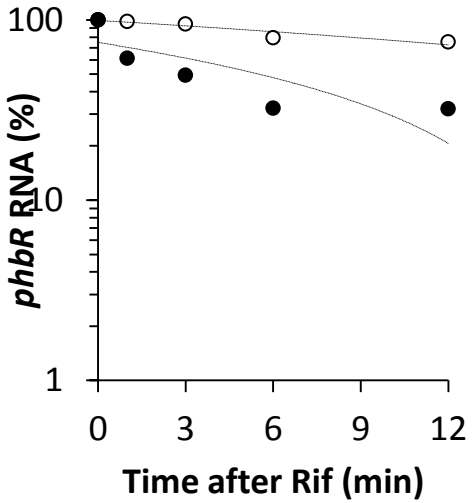


Fig. 5. Stability of *phbR* mRNA in the wild type UW136 strain (black circles) and in the *rsmA* mutant (empty circles). The line represents a tendency of the points.

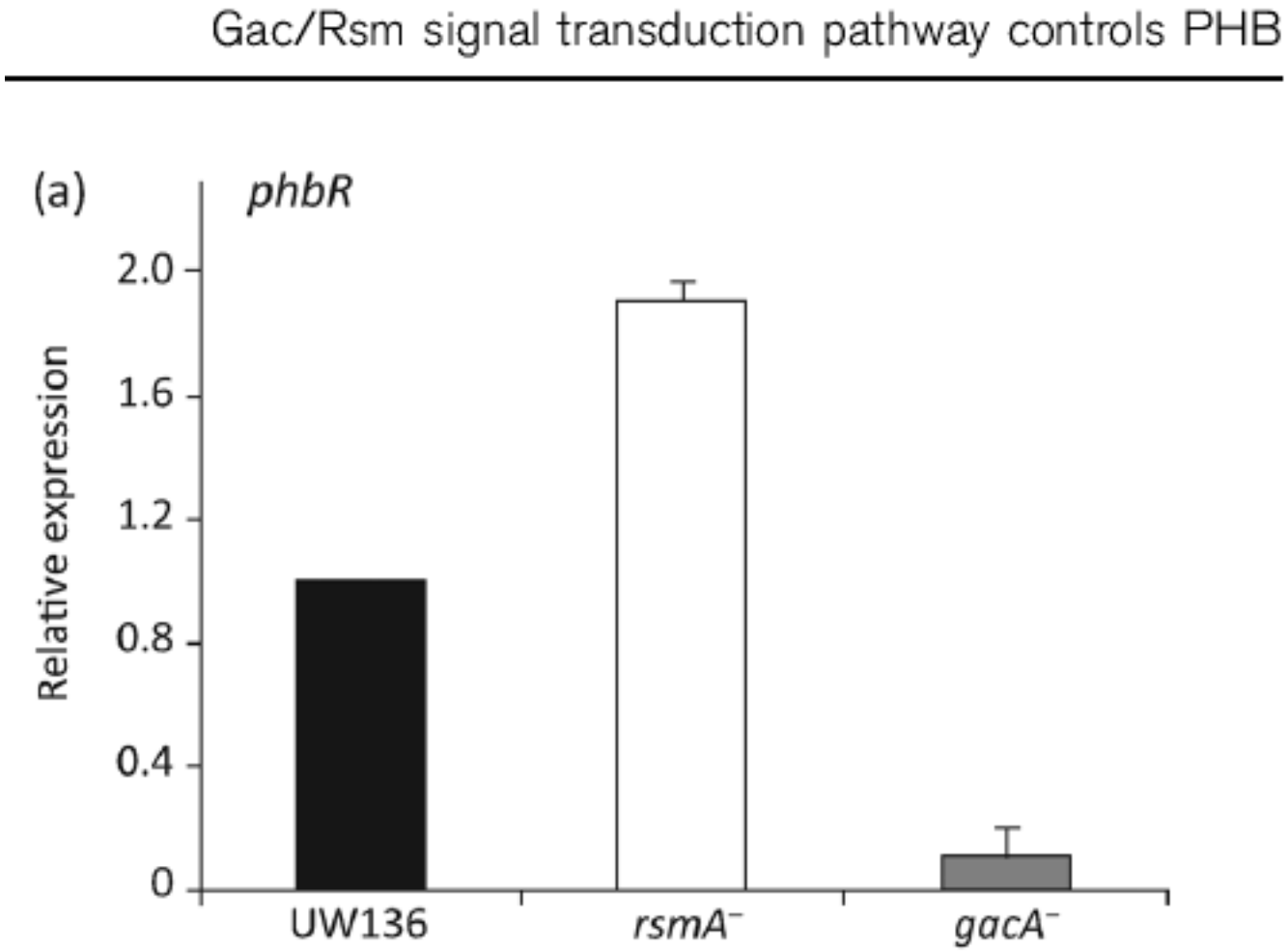


Fig. 6. qRT-PCR analysis of *phbR* transcript using RNA isolated from cultures of *A. vinelandii*. UW136, Ah*rsmA* and Ah*gacA* strains

Hernandez-Eligio et al 2012, Microbiology 158: *in press*

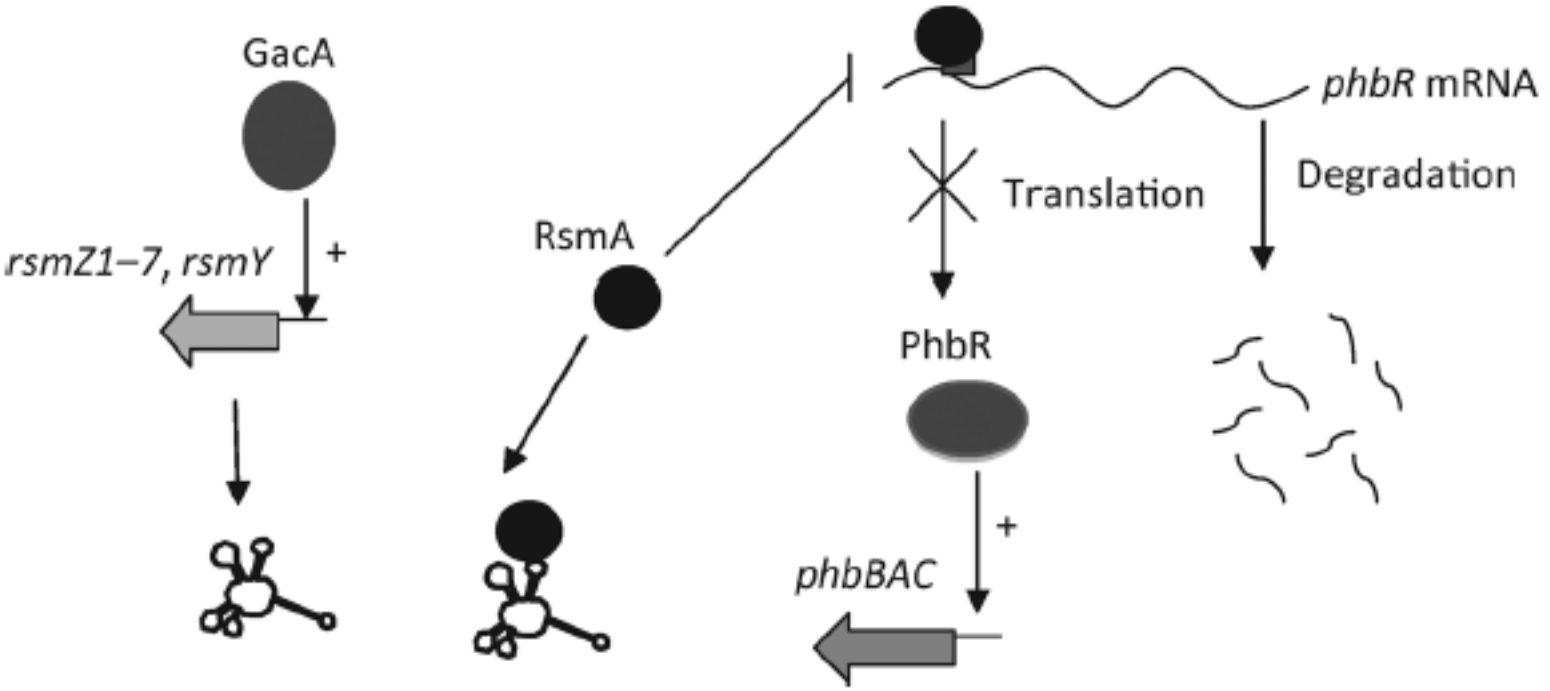


Fig. 7. Model for the post-transcriptional control of PhbR, the transcriptional activator of the *phbBAC* PHB biosynthetic operon by the Gac-Rsm system in *A. vinelandii*. GacA activates transcription of one *rsmY* and seven *rsmZ* genes, whose products, the small Rsm RNAs, interact with the RsmA protein. This interaction prevents the binding of RsmA to the *phbR* transcript, allowing its translation. Degradation of the *phbR* transcript occurs in the presence of RsmA protein.

Grupo:

Daniel Segura, Cinthia Núñez, Mildred Castellanos, Soledad Moreno, Josefina Guzmán, *Renato León, Alberto Hernández, Yanet Romero, Miguel Cocotl, *Claudia Velazquez, Deborah Yanajara, *Jade Serrano, *Fernando Bonilla, *Viridiana García, Armando Hernandez, Miguel Mejía, Luis Felipe Muriel, Libertada Adaya, Deborah Ramirez, Elva Yadira Quiroz, Adan Trejo.

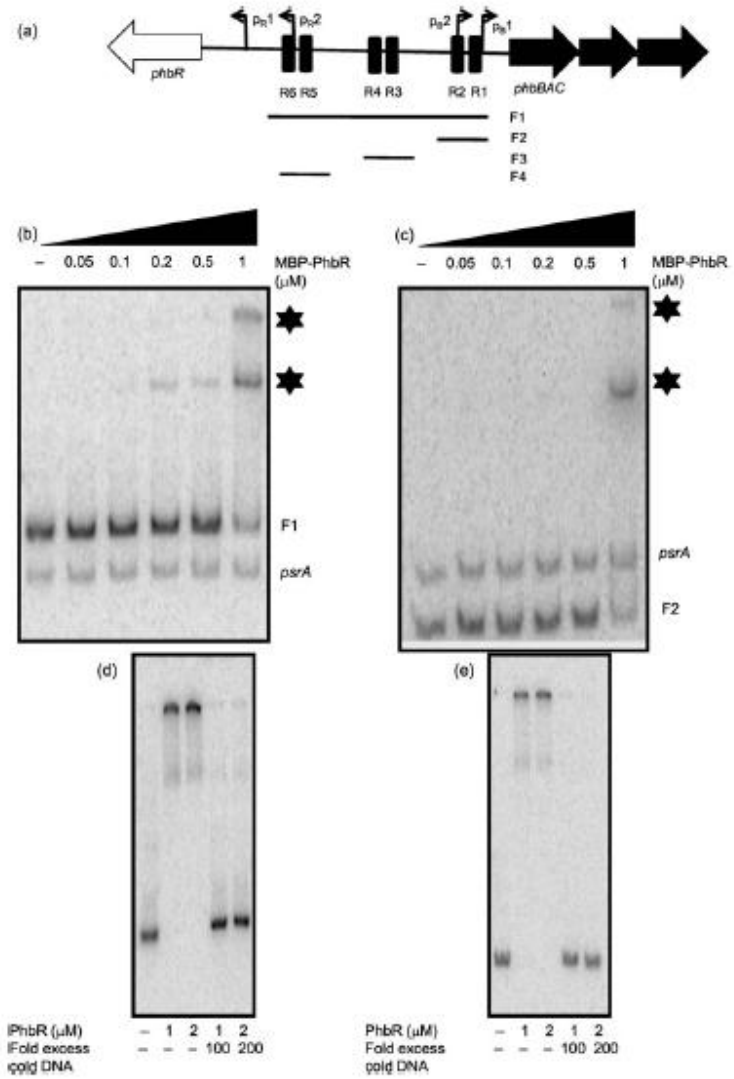
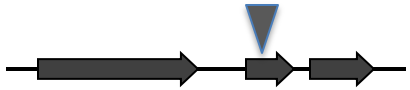


Fig. 5. PhbR protein binds the *phbB* promoter region. (a) Schematic representation of the *phbR–phbB* intergenic region, showing the position of the *phbB* $p_{B}1$ and $p_{B}2$ and *phbR* $p_{R}1$ and $p_{R}2$ promoters (transcription start sites) and the proposed PhbR-binding sites R1 to R6 (Peralta-Gil *et al.*, 2002). The PCR F fragments used for the EMSA for the binding of PhbR are depicted. (b, c) EMSA showing the binding of various concentrations of PhbR (0.05–1 μM) to the F1 fragment (b) or to the F2 fragment (c). Stars show PhbR–DNA complexes. (d) Competition assay for PhhR–F1 complex formation to verify the binding specificity. Competition reactions used specific unlabelled competitor (F1 fragment) at the concentrations shown. (e) Competition assay for PhhR–F2 complex formation to verify the binding specificity. Competition reactions used specific unlabelled competitor (F2 fragment) at the concentrations shown.

Efecto de la inactivación del gene *rsmA* sobre la síntesis de PHB



rsmA

Cepa	**Contenido PHB [μg (μg proteína)-1]**
UW136	245 ± 15
rsmA-	286 ± 20