

**Study of the response regulator Rrp1 reveals its regulatory role on chitobiose utilization
and virulence of *Borrelia burgdorferi***

Ching Woon Sze¹, Alexis Smith³, Young Hee Choi², Xiuli Yang³, Utpal Pal³, Aiming Yu², and
Chunhao Li^{1*}

Department of Oral Biology, The State University of New York at Buffalo, Buffalo, New York¹,

*Department of Pharmaceutical Sciences, The State University of New York at Buffalo, Buffalo, New
York²,*

*Department of Veterinary Medicine, University of Maryland and Virginia-Maryland Regional College
of Veterinary Medicine, College Park, Maryland³*

Running title: Regulation of chitobiose utilization in *Borrelia burgdorferi*

Key words: Lyme disease/ *Borrelia burgdorferi*/ chitobiose/ Rrp1/ c-di-GMP/ BosR-RpoN-RpoS

* Corresponding author. Mailing address: Department of Oral Biology, SUNY at Buffalo, 3435 Main
St., Buffalo, NY 14214-3092

Electronic mail address: cli9@buffalo.edu; phone: (716) 829-6014; Fax: (716)-829-3942

Table S1. Oligonucleotide primers used in this study.

Primer	Description	Sequences
P ₁	mutagenesis of <i>bb0419</i> (F)	5'-GAAGCAGAGAATCAGAAGC-3'
P ₂	mutagenesis of <i>bb0419</i> (R)	5'-CCTTTAACACTTGGCCTTC-3'
P ₃	Kan (F)	5'- <u>TCTAGATA</u> AATACCCGAGCTTCAAG-3'
P ₄	Kan (R)	5'- <u>TGGCCATTAGAAAA</u> ACTCATCGAGC-3'
P ₅	complementation, upstream of <i>bb0420</i> (F)	5'- <u>GGATCCTTGTAAAA</u> ACCCTGGTGG-3'
P ₆	complementation, upstream of <i>bb0420</i> (R)	5'-CTTTAATTATCATTTCACGTTATTTT AAAATTCCT-3'
P ₇	complementation, <i>bb0419</i> (F)	5'-AGGGAATTTTAAAATAACGTGGAAAT GATAATTAAAG-3'
P ₈	complementation, <i>bb0419</i> (R)	5'- <u>GGATCCTTAATATCTAA</u> ACTGATTTTC-3'
P ₉	pCisCom 5' arm (F)	5'-ATGGGTGTTTTAGATAAGATTAAAC-3'
P ₁₀	pCisCom 5' arm (R)	5'- <u>CCATGG TCTAGATTA</u> ATTTCTATTA ATATTATTAAG-3'
P ₁₁	pCisCom 3' arm (F)	5'- <u>CCATGGT</u> TAATTGTCACCATCAAC-3'
P ₁₂	pCisCom 3' arm (R)	5'- ATGTTTAAAGTTATCAAATG-3'
P ₁₃	<i>aacC1</i> (F)	5'- <u>TCTAGA GGATCC CTGCAGTA</u> ATAC CCGAGCTTCAAG-3'
P ₁₄	<i>aacC1</i> (R)	5'- <u>GAATTC CCATGGT</u> TAGGTGGCGGT ACTTGGGTC-3'
P ₁₅	<i>chbC</i> rescue in <i>rrp1^{mut}</i> , <i>flgBp</i> (F)	5'- <u>GCATGCTACCCGAGCTT</u> CAAGGAAG-3'

P ₁₆	<i>chbC</i> rescue in <i>rrp1^{mut}</i> , <i>flgBp</i> (R)	5'- <u>CATATGATGGAAACCTCCCTCATT</u> TA-3'
P ₁₇	<i>chbC</i> rescue in <i>rrp1^{mut}</i> , <i>chbC</i> (F)	5'- <u>CATATGATGAATTTTCAAGATTTTATT</u> GAAACTAC-3'
P ₁₈	<i>chbC</i> rescue in <i>rrp1^{mut}</i> , <i>chbC</i> (R)	5'- <u>GCATGCCTATTCTTTTTCTGCAAAAAG</u> -3'
P ₁₉	qRT-PCR, <i>rpoS</i> (F)	5'-ACCTATCTCCTGCTCAGTATATAA-3'
P ₂₀	qRT-PCR, <i>rpoS</i> (R)	5'-CAAGGGTAATTTTCAGGGTTAAAAG-3'
P ₂₁	qRT-PCR, <i>bbb04</i> (F)	5'-GCTGGACAATCAGATTGGATAC-3'
P ₂₂	qRT-PCR, <i>bbb04</i> (R)	5'-TCCTAAAGAACTTTGAATAGCA-3'
P ₂₃	qRT-PCR, <i>bosR</i> (F)	5'-CCTGTATTATTGAAAAATTTAACAT CAG-3'
P ₂₄	qRT-PCR, <i>bosR</i> (R)	5'-GACTTGATTGCATTTATTGCAT-3'
P ₂₅	qRT-PCR, <i>flaB</i> (F)	5'-CCCTGAAAGTGATGCTGGTGTG-3'
P ₂₆	qRT-PCR, <i>flaB</i> (R)	5'-CATATTCAGATGCAGACAGAGG-3'

The underlined sequences are the engineered restriction cut sites for DNA cloning.

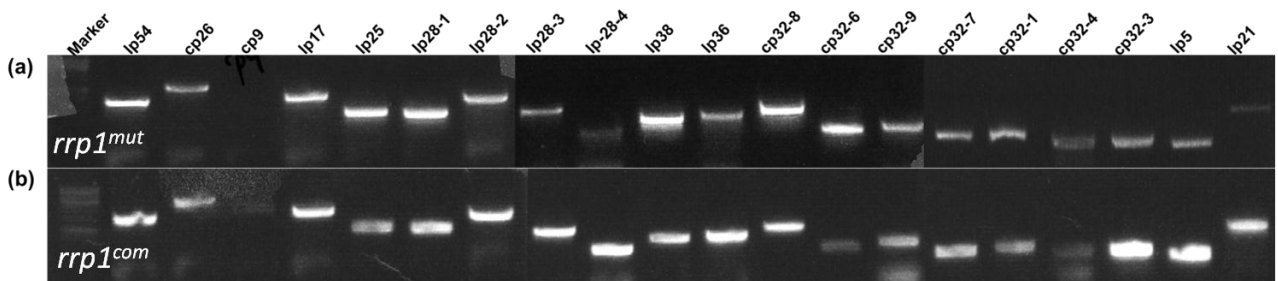


Fig.S1. Detection of plasmid contents in *rrp1^{mut}* (a) and *rrp1^{com}* (b) strains by PCR. The primers for PCR were described as before (1). The parental strain A3-68 lacks lp56 (2).

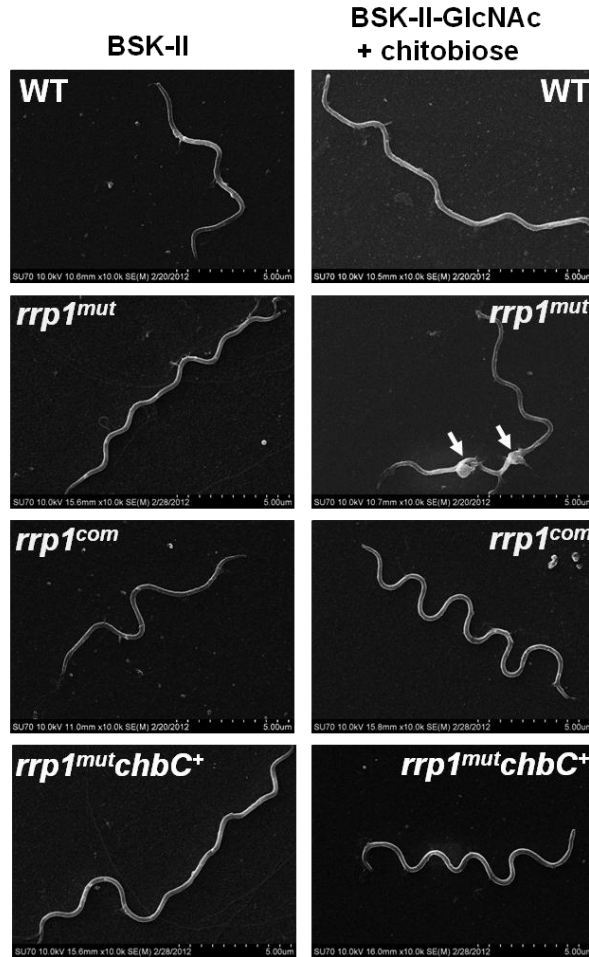


Fig.S2. Scanning electron microscope analysis. The wild type (WT), *rrp1^{mut}*, *rrp1^{com}*, and *rrp1^{mut}chbC⁺* strains were cultured in both normal BSK-II medium (left panel) and BSK-II – GlcNAc + chitobiose medium (right panel). Stationary phase cells (Day 8) were subjected to scanning electron microscope analysis. Arrows point to membrane blebs.

Reference List

1. **Elias AF, Stewart PE, Grimm D, Caimano MJ, Eggers CH, Tilly K, Bono JL, Akins DR, Radolf JD, Schwan TG, Rosa P.** 2002. Clonal polymorphism of *Borrelia burgdorferi* strain B31 MI: implications for mutagenesis in an infectious strain background. *Infection and immunity* **70**:2139-2150.
2. **Rego RO, Bestor A, Rosa PA.** 2011. Defining the plasmid-borne restriction-modification systems of the Lyme disease spirochete *Borrelia burgdorferi*. *Journal of bacteriology* **193**:1161-1171.