Supplemental Information

Genetic disruption of the bacterial *raiA* motif noncoding RNA causes defects in sporulation and aggregation

Lucas W. Soares[a], Christopher G. King[b], Chrishan M. Fernando[b], Adam Roth[c], and Ronald R. Breaker[b,c,d,1]

[a]Department of Microbial Pathogenesis, Yale University, New Haven, CT 06536, USA;

[b]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511-8103, USA;

[c]Howard Hughes Medical Institute, Yale University, New Haven, CT 06511-8103, USA;

[d]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06511-8103, USA

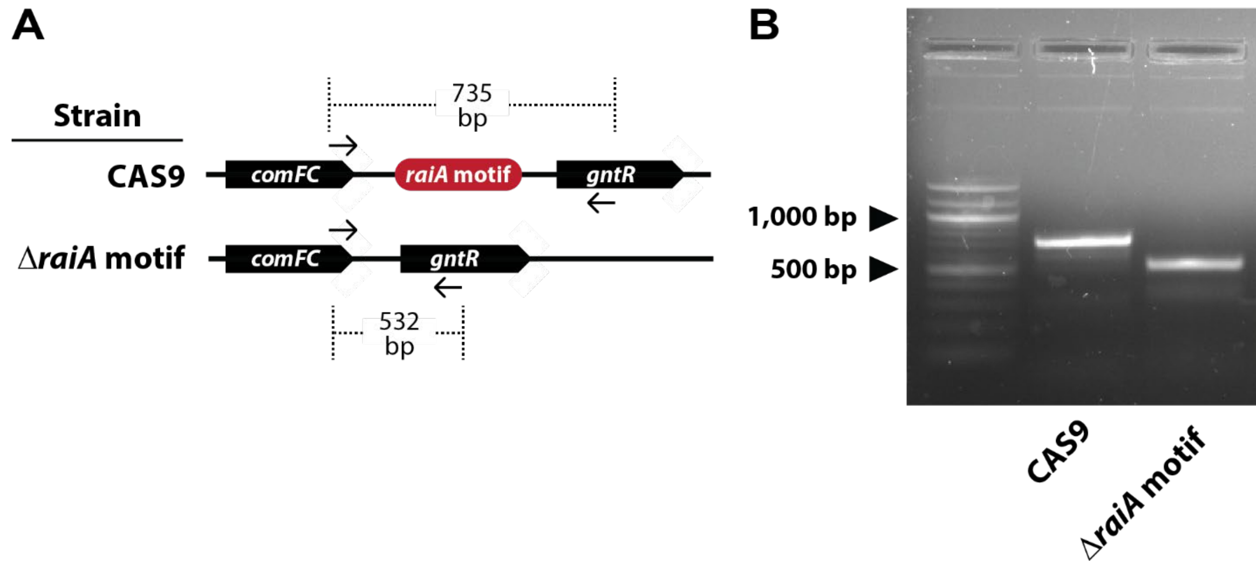

Figure S1. Confirmation of genetic deletion of the *raiA* motif gene by PCR. (**A**) Genetic map of the *raiA* locus, depicting the flanking genes. Direction of all three genes is 5′ to 3′ from left to right. Sizes of the PCR amplification products are indicated for each strain. See **SI Appendix, Table S3** for primer list. (**B**) Agarose (1%) gel electrophoresis of PCR reactions of genomic DNA from the CAS9 and Δ*raiA* motif strains, respectively. First lane was loaded with a 100 base-pair (bp) DNA ladder. The sizes of the PCR products are consistent with the expected PCR product sizes for the two strains.

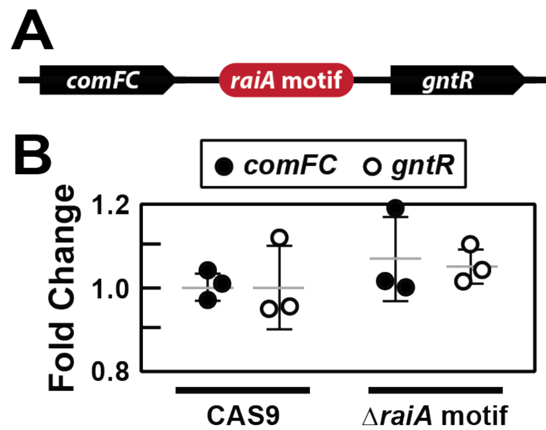

Figure S2. Assessment of mRNA levels for genes flanking the *raiA* motif gene in CAS9 and Δr*aiA* motif strains of *C. acetobutylicum* ATCC 824. (**A**) Depiction of the genes flanking the *raiA* motif gene from *C. acetobutylicum*. Direction of all three genes is 5′ to 3′ from left to right. (**B**) Quantitation of mRNAs for the *comFC* and *gntR* genes, located immediately upstream and downstream of the *raiA* motif gene, respectively, was achieved by qRT-PCR using the appropriate primers (see ***SI Appendix*****, Table S3**). Fold change values were established by first recording ct values from the qRT-PCR process. The average ct values reflecting mRNA levels of genes of interest in the CAS9 strain were normalized to a value of 1 'fold change', and each individual ct value was divided by the average ct value for the corresponding mRNA. The fold change values for mRNA levels in the Δ*raiA* motif strain were established by dividing the ct value for each point by the average ct value for the same gene in the CAS9 strain.

Table S1. List of bacterial strains used in this study.

Strain	Characteristics	Source or Reference
E. coli TOP10		Invitrogen
C. acetobutylicum ATCC 824		ATCC
CAS9	*pyrE*::*pXyl_cas9*	*a*
CAS9*ΔraiA* motif	*pyrE*::*pXyl_cas9, ΔraiA*	This study
raiA motif rescue	*pyrE*::*pXyl_cas9*, *ΔraiA*, praiARescue	This study

a. T. Wilding-Steele, Q. Ramette, P. Jacottin, P. Soucaille, Improved CRISPR/Cas9 tools for the rapid metabolic engineering of *Clostridium acetobutylicum*. *Int. J. Mol. Sci.* **22**, 3704 (2021).

Table S2. List of plasmids used in this study.

Name	Characteristics	Source or Reference
pINT*tcas9*	*Cm*^{r}, *repL*, *pyrE*::*pxyl_cas9*	*a*
pgRNAraiAKO	*Cm*^{r}, *repL*, *ΔraiA* motif	This study
praiARescue	*Cm*^{r}, *repL*, *raiA* motif	This study

a. T. Wilding-Steele, Q. Ramette, P. Jacottin, P. Soucaille, Improved CRISPR/Cas9 tools for the rapid metabolic engineering of *Clostridium acetobutylicum*. *Int. J. Mol. Sci.* **22**, 3704 (2021).

Table S3. List of oligonucleotides used in this study.

Identifier	Sequence	Description
LWS57	TAATACGACTCACTATAGGGTTAAGTTAGGTTTGTGGTTGAAAGTCGATGCCAGTCGCAGGCAAAACGATCCACGTAAGTTAAACAAAGTTTTAATGAGCATGGTGCGGCTTAGAAGTAAGTCCTGCCGCTTTAGGCGAGAGTATTAGTAGTGAGAGGGTAATTCCGGGTAGCGAAACTTCCAGCAGGCGAGTGTGGGGTCAAAGACCAGGTCAACTAACTTA	*raiA* motif RNA in vitro transcription template
LWS61	TAATACGACTCACTATAGGGTTAAGTTAGG	Primer for amplifying LWS57
LWS62	TAAGTTAGTTGACCTGGTCTTTGACC	
LWSP22	CCCTCTCACTACTAATACTCTCGCCTAAAGCGGCA	Northern blot probe for *raiA* motif RNA
LWSP24	GCCCCTGCAGTACCATCGGCGCTTTGGTTCTTAAC	Northern blot probe for 5S rRNA
LWS147	TGGAAGTTTCGCTACCCGGAAT	Primer for making the *raiA* motif cDNA for qRT-PCR
LWS148	GGTTGAAAGTCGATGCCAGTCG	
LWS161	AGGGCTACACACGTGCTACAAT	Primer for making the 16S cDNA for qRT-PCR
LWS162	ACTAGCAACTCCGGCTTCATGT	
LWS141	TTGCAACACCACCAAGTTGAGC	Primers for making the *fabZ* cDNA for qRT-PCR
LWS142	CCAGGGAAAAGAGCAGTGGGAT	
LWS143	CAAAGCCCGGCATTGTAACAGT	Primer for making the *nadE* cDNA for qRT-PCR
LWS144	GACTTGACTCCACCCTTGCTCT	
LWS79	TACGATGGAGAACCTCCTCTTT	Primers for PCR screening of *ΔraiA* motif strains
LWS80	TGTGTTTACTACGGGAGCGA	
LWS125	TGTTGGAACAGAAATAGCTGGATGT	Primers for screening of *cas9* integration based on primer PS1 from *a*
LWS126	ACCAGAAGATAAGGATGCTCTAGTTGA	
LWS137	TTTCGCCCTATAGTGAGTC	Primers for amplification of a segment of pgraiAKO without the HDR and gRNA region for Gibson assembly
LWS138	TAATGATCCAGATCCGTG	
LWS266	CTCACGGATCTGGATCATTAAAGACATTTGTCCATAGAC	Primers for production of praiARescue, amplifies the native *raiA* motif loci with ~200 additional nucleotides on each end to capture promoter and terminator
LWS267	CGACTCACTATAGGGCGAAATTACAAACTAAGGACCAAATAG	

		regions. The primers also carry an additional 20 nucleotides on each primer to permit Gibson assembly with the appropriate DNA.
LWS285	GAAAGCTTAGAAGCTAAAAATTCGC	Primers for the amplification of the *comFC* genomic region for qRT-PCR
LWS286	TGGTGAGGTATTAGCGGAACT	
LWS283	TCTGCTTTTAAGGATGTTAATGAATTTGT	Primers for the amplification of the *gntR* genomic region for qRT-PCR
LWS284	TCTCATCTTGCAAGTCAATACAAAGT	

a. T. Wilding-Steele, Q. Ramette, P. Jacottin, P. Soucaille, Improved CRISPR/Cas9 tools for the rapid metabolic engineering of *Clostridium acetobutylicum*. *Int. J. Mol. Sci.* **22**, 3704 (2021).