Supplementary File S1: Vector Construction

pMC381: A 1000 bp fragment containing the region upstream of CD1492 was amplified using primers oMC911 and oMC912. A second fragment (1109bp) was amplified using primers oMC913 and oMC914, and subsequently spliced to the 1000 bp fragment and amplified using primers oMC911 and oMC914. The resultant fragment was cloned as *Not*I into pMTL7315.

pMC386: A 2885 bp fragment containing the *CD1492* coding sequence and flanking sequence was amplified using primers oMC897 and oMC898, and cloned as *Bam*HI/*Pst*I into pMC211.

pMC538: A fragment containing the same sequence as pMC386 was synthesized (Genscript) to containing a site-directed substitution at amino acid position 668 in the CD1492 protein sequence (H668A).

pMC539: The 2.9 kb *CD1492* sequence from pMC538 was cloned as *Bam*HI/*Pst*I into pMC211.

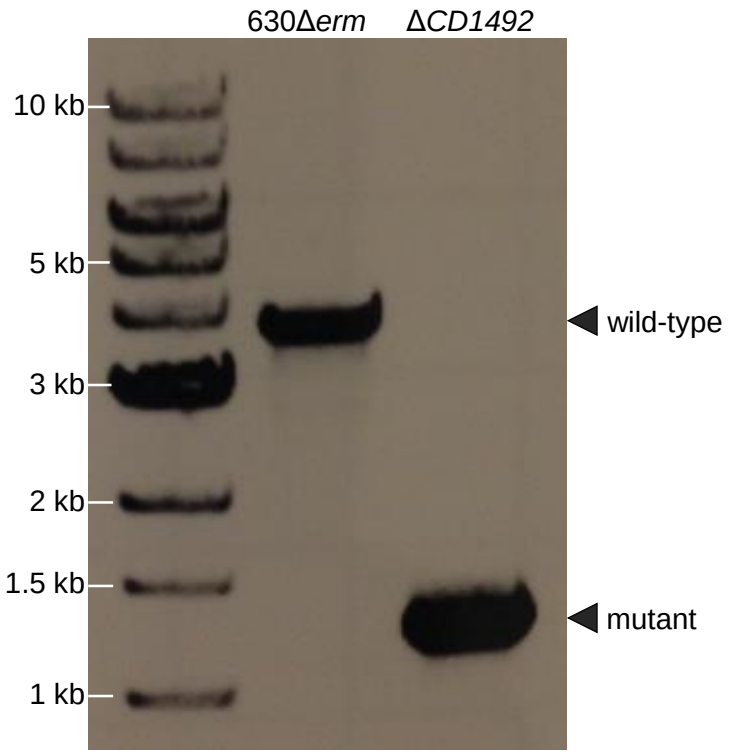

Figure S2. Confirmation of gene deletion for sensor histidine kinase, *CD1492*. PCR amplification across the 5' and 3' flanking regions of *CD1492* (oMC937/oMC914) from *C. difficile* strains, demonstrating deletion of *CD1492* in strain MC674.

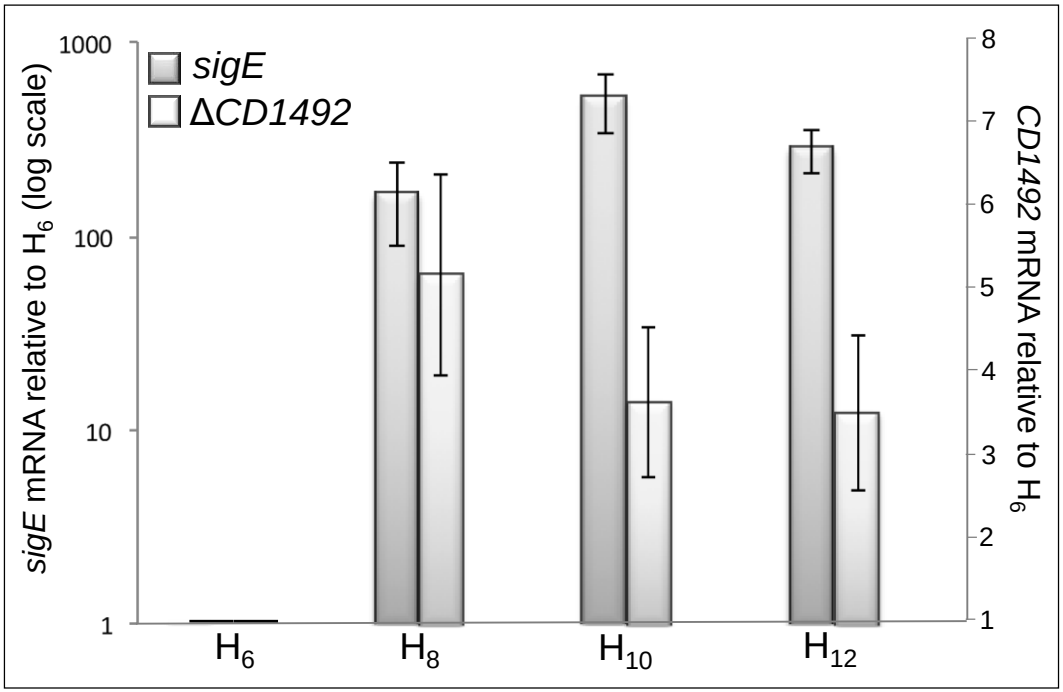

Figure S3. Expression of *CD1492* coincides with the onset of sporulation. Transcriptional analysis of *CD1492* and the early sporulation sigma factor, *sigE*, in strain 630Δ*erm* grown on 70:30 agar medium for 12 hours. The means and standard error of the means for at least four biological replicates are shown.

	630Δ*erm* p*Pcpr* (MC282)		630Δ*erm* p*Pcpr::CD1492* (MC587)		*CD1492* p*Pcpr* (MC729)		*CD1492* p*Pcpr::CD1492* (MC730)	
Nisin (1 µg/ml)	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
Transcript								
CD1492	1.0 ± 0.0	0.9 ± 0.4	**3.0 ± 0.5**	5.8 ± 2.1	0.0 ± 0.0	0.0 ± 0.0	**3.2 ± 0.7**	5.3 ± 2.0
CD1579	1.0 ± 0.0	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.3	1.1 ± 0.2	1.3 ± 0.2	1.3 ± 0.2
CD2492	1.0 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.1
sigF	1.0 ± 0.0	0.9 ± 0.0	**0.7 ± 0.1**	0.8 ± 0.1	1.5 ± 0.2	2.0 ± 0.5	1.4 ± 0.7	1.6 ± 0.7
sigE	1.0 ± 0.0	1.0 ± 0.3	1.1 ± 0.2	1.3 ± 0.3	2.0 ± 1.0	3.2 ± 1.2	4.3 ± 3.8	4.2 ± 3.3
sigD	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.2	1.3 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	1.3 ± 0.2	1.3 ± 0.2
fliC	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	1.3 ± 0.2	1.2 ± 0.1
rstA	1.0 ± 0.0	0.9 ± 0.0	0.8 ± 0.1	**0.7 ± 0.1**	0.9 ± 0.2	1.0 ± 0.2	1.3 ± 0.1	1.3 ± 0.1
tcdA	1.0 ± 0.0	0.9 ± 0.0	**0.7 ± 0.1**	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	1.3 ± 0.3	1.5 ± 0.3
tcdB	1.0 ± 0.0	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.0	1.0 ± 0.2

Figure S4. Transcriptional analyses of *CD1492* complementation. qRT-PCR analysis of gene transcription for 630Δ*erm* p*Pcpr* (MC282, vector control), 630Δ*erm* p*PcprA::CD1492* (MC587), *CD1492* p*Pcpr* (MC729, vector control) and *CD1492* pP*cprA::CD1492* (MC730) grown on 70:30 sporulation agar plates supplemented with 2 µg ml^{-1} thiamphenicol with or without 1 µg ml^{-1} nisin (+/-). Primers for qRT-PCR are listed in Table 2. Results were normalized to the control strain MC282 grown without nisin, for each independent experiment (first column). Statistical comparisons were made between MC282 and MC587 or MC729 and MC730 from the same growth conditions. The means and standard error of the means for at least three biological replicates are shown. Bold type indicates $P \leq 0.05$ by Student's two-tailed *t* test.

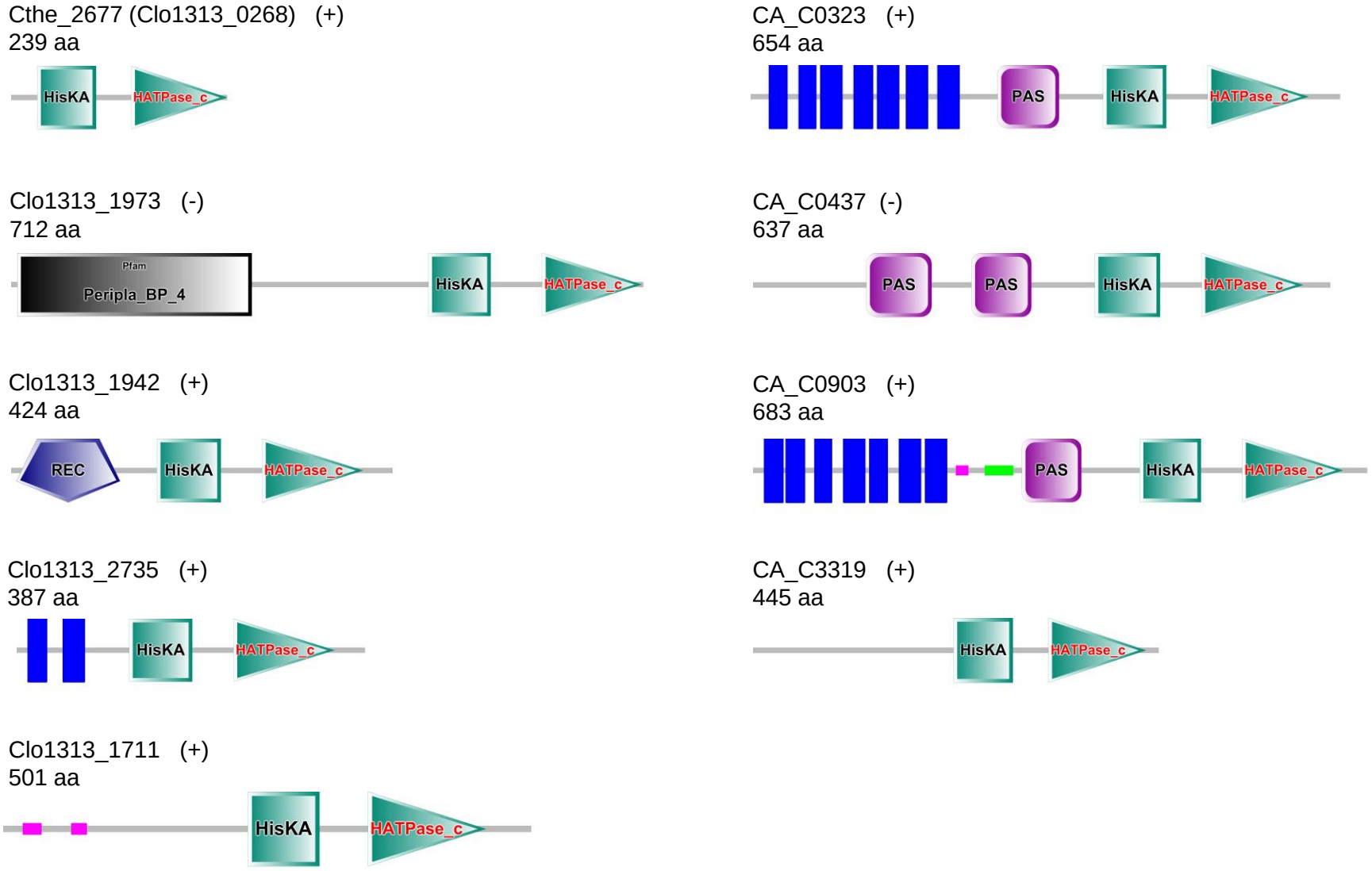

Figure S5. Sporulation sensor phosphotransfer proteins of *R. thermocellum* and *C. acetobutylicum*. Protein domains were predicted using the SMART analyzer at http://smart.embl-heidelberg.de All of the proteins contained HisKA and HATPase domains that are characteristic of sensor histidine kinases. Predicted transmembrane segments are shown in blue, PAS sensor domains in purple, coiled-coil regions in lime green and low-complexity regions are shown in pink. Other detected regions include a CheY-like receiver domain in Clo1313_1942 and a predicted periplasmic binding region in Clo1313_1973 (55, 56). (+) and (-) indicate positive and negative effects of each protein on sporulation, respectively (32, 33).