Fig. S1. Procedures for construction of *crgA* null mutants and complemented strains. (A) Disruption of *crgA* with split-marker strategy. In round 1, primer pairs 5f/5r and 3f/3r were used to amplify 5' and 3' flanks, and the selectable marker was amplified from plasmid pCSN44 with primer pair hF/hR. In round 2, 5' and 3' flanks were fused to 5'-hp and hp-3' fragments by two separate overlap PCR using primer pairs 5f/hf and hr/3r, respectively. In round 3, the two deletion cassettes (5'-hp and hp-3' fragments) were co-transformed into protoplasts, such that part of *crgA* is replaced by *hph* through homologous recombination. (B) Construction of complemented strains with split-marker strategy. In round 1, primer pairs Cu/Cd and Cf/Cr were used to amplify 5' and 3' regions of *crgA* gene by PCR and two fragments (*crg* and *rgA*) with partial overlap were obtained. In round 2, the two partially complemented fragments (*crg* and *rgA* through homologous recombination.

Fig. S2. Sequence alignment for crgA and its deduced protein from strain (-) of *B. trispora* with those from (+) strain. (A) Nucleotide sequence alignment for crgA from the opposite mating type strain of *B. trispora*. The nucleotides are numbered in 5' to 3' direction and the identical bases are shaded in gray. The boxed letters of GTG and TAA represent the starting codon and stop codon, respectively. The doubly and singly underlined sequences indicate the putative TATA box and exons of gene crgA, respectively. Sequence marked by dashed lines and wavy line denote the putative promoter and intron, respectively. (B) Sequence alignment of deduced protein (CrgA) from the opposite mating type strain of *B. trispora*. The identical amino acid

residues are marked with a grey background. The doubly underlined and boxed sequences indicate the RING-finger (RF1 and RF2) and the disrupted LON domains, respectively. Heptapeptide (HP) and isoprenylation (IP) sequences are marked by dash line and solid line, respectively.

Fig. S3. Isolation of *crgA* null mutants on the solid media and PCR analysis demonstrating the replacement of *crgA* gene by homologous recombination. (A) The transformants of *B. trispora* was grown on the selection media containing 200 µg/ml hygromycin B and 0.1% Trition×100, compared with no growth for the WT strain. (B) Four pairs of primers were designed to characterize the *crgA* locus from the wild-type and mutant strains. P1 and P2 were used for amplification of the middle sequence of *crgA* from WT strain. Primer pairs P3/P4, P5/P6 and hR/hF were used to confirm knockout of *crgA*. (C) Electrophoretic analysis of PCR amplicons from WT and three *crgA* null mutants using primers indicated in panel B. There are four lanes from left to right, corresponding to WT and three mutant strains, respectively. M = DNA ladder.





Round 3



Fig. S1A



Fig.S1B

crgA(-) crgA(+)	ggraattaagctatgcaccgcagtatagtcaagattatacagaaaggagcatctgcagagaagcccaagttgcctggagagacctaggagaggcttctgagg ggaaattaagctatgcaccgcagtatagtcaagattatacagaaaagaggatctgcagagaaggccaagttgcc.ggaagacctaagaaggcttctgagg	100
crgA(-) crgA(+)	GAGTACATTCGTTCATTGTTGCTATATGTAGCACAGAACCTTTTGATAGCTTTGCCAAAATTCAGACACAAGCCAAGGCCTTTGGGTTTGATACGTCCA GAGTACATTCGTTCGTTGTTGCTATATGTAGCACAGAACCTTTTTGATAGGTTTGCCAAAATTCAGACACAAGCCAAGGCCTTTGGGTTTGATACGTCCA	200 199
crgA(-) crgA(+)	AAGACACAATCATCGGCTATCTAGCCCAGAATGGCTTCC.ATTCGATCTGGACAGCTCATAGACCAAAGCTGACGGATAGACCCAAGGCAAAACGACTGG AAGACACAATCATCGGCTATCTAGCCCAGAATGGCTTCCCATTCGATCTGGACAGCCAAAGCCAAAGCCGATAGACCCAAGGCAAAACGACTGG	299 299
crgA(-) crgA(+)	ATTGGGCACTTACCCACACACATGGACCAGTTACCAGTAGGAGGGGTGCATCTGGTCGGATGAGTCCGTTCCGAGTGGAGAAACAACGACGGTGGAGAAACAACGACGGGGGGGG	399 399
crgA(-) crgA(+)	TCCC.GGGTAATCTGTAAGAAGGGGG.ACACGCTAGAGGAGAGG	497 499
crgA(-) crgA(+)	GTTTTGGTCCCTTGGTGGCAGTTGGAGAAGAAGATTGGCTGCCGAAAAACATATCGTCCAAAATGTGAAGGAATTGGCAGCCCCCTCATCCATC	597 599
crgA(-) crgA(+)	GACACTT, AGTCGARAGTATGCCARATAGATGTGCAGCGCTTCTATARAGGCGARAGGCGGGCGGGCATACARGATACTARARARACCARTTTCTTATTTA GACACTTTAGTCGARAGTATGCCARATAGATGTGCAGCTGTCATARAGGCGARAGGCGGGGGGGGGG	696 699
crgA(-) crgA(+)	TTTTAATAAAAGAAGACACTTTTTTGATCTGTTTTGCATCATGGACGTGTTTTGGCCATGCTTTCAAACTTTTGTTTTCAGTTTGGATTTAATTTAATTT TTTTAATAAAGAAGACACTTTTTTTGATCTGTTTTGCATCATGGACGTGTTTTGGCCATGCCTTTCAAACTTTTGTTTTCAGTTTGGATTTAATTTAATTT	796 799
crgA(-) crgA(+)	CACCGARA. ACCETTIGATAGGTAATGTATGTCGGGGTTGGTTTTGAATCGTGATAGATGTAACCGGGACACTTCTCTTTTCTGATCGGGAAACTGTTG CACCGARAGACGCTTTGATAGGTAATGTATGTCGGTGTTGGTTTTTGAATCGTGATAGGTGAAACTGGGACACTTCTCTTTTCTGATCGTGAAACTGTTG	895 899
crgA(-) crgA(+)	${\tt transcerrate} aratematics are consistent to a construct to the transcerre transcere transcerre transcerre transcerre$	995 999
crgA(-) crgA(+)	TTCTG-TCTCCCAAAAAAAAAA.GCCTACGTTTTGAGTAGGTCGATCCACTAATAAAGAGCAAGAGAGAG	1094 1099
crgA(-) crgA(+)	TCAAATTTATACACTTTTGETAATTATCGATCATGAAGAAAAGAGGATTTCCCTCAGAAGGGGGGGG	1194 1199
crgA(-) crgA(+)	$\label{eq:construct} a construct a const$	1292 1299
crgA(-) crgA(+)	$\label{eq:transformation} regarded re$	1390 1399
crgA(-) crgA(+)	ATTTCAAAAATAGTAAATGCTAAAAATGGAGTAAATTGCTGAGAGAGA	1490 1499
crgA(-) crgA(+)	AAATATATAAAAATGAATTTTTTTTTTTTTTTTTTTTT	1588 1599
crgA(-) crgA(+)	LAIA 60X ACATTCCTGTAAAATTCCTTCTTTTTTTTTTTTTTTTTT	1688 1699
crgA(-) crgA(+)	artetattetatteatteatteattetattteaaggaaaaaaaa	1787 1799
crdy(-)	${\tt GCATACAACAACAACAACAACGATCCCCCCGCCACCACGTCGACACTTACCTGTAGGACAATTCAATAAGAAGCAAAACCAGATCGATGTATTTTCACCCTCTGTGCACAACAACAACAACAACAACAACAACAACAACAACAAC$	1887 1899
crgA(-) crgA(+)	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	1987 1999
crgA(-) crgA(+)	tcatcacaacaatgtatatcacctacttgtgatcgcctgcatacaatcgcacttcaaccgagtgtcactattcgggcatcgcaagccattgtagtcagtg tcatcacaacaatgtatatcacctacttgtgatcgcctgcatacaatcgcacttcaaccgagtgtcactattcaggcattgcaagccattgtagtcagtg	2087 2099
crgA(-) crgA(+)	cagaage at ctcgae act core cast core cases to care core cases and core core cases and core cases act core cas	2187 2199
crgA(-) crgA(+)	$\label{eq:construct} a transformation of the transformation of transformation of the transformation of tra$	2287 2299
crgA(-) crgA(+)	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	2387 2399
crgA(-) crgA(+)	TGTTG&TTGGCAACCTGGCCTTTCCTCATGTCAAAATGTGCCATTCATGTGTTTGAACCTCGCTATCGTCTTATGCTTCGTCGTCATGCAGCGTCCAACCG TGTTGATTGGCAACCTGGCCTTTCCTCATGTCAAATGTGCCATTCATGTGTTTGAACCTCGCTATCGTCTTATGCTTCGTCGTATCATGCAGCCCAACCG	2487 2499
crgA(-) crgA(+)	TOGTOGATTTGCCATGTGTATTGCTCGTCGAAACAGATCAGAAGGGCAAGGTCCATTTACGAATATGGCACTATGCTTGAATTGACCCATGTTCAAACT TCGTCGATTTGCCATGTGTATTGCTCGTCGAAACAGATCAGAAGGGCAAGGTCCATTTTACGAATATGGCACTATGCTTGAATTGACCCATGTTCAAACT	2587 2599
crgA(-) crgA(+)	TTGCCTGATGGTCGTTCTTTGGTAGAAGCCATTGGCTCGCATCGCTTCAAGGTCCTCGACTATGAATTGACAGATGGTATCATATGGCTTCCATTGAAA TTGCCTGATGGTCGTTCTTTGGTAGAAGCCATTGGCTCGCATCGCTTCAAGGTCCTCGACTATGAATTGACAGATGGTATCATATGGCTTCCATTGAAA	2687 2699
crgA(-) crgA(+)	GANTIGATGATATIGATGGTGAGCAAGAAAACATGCITGAACGACAACAGATCITGAGAGCCAGTGCGTCTCGTGCACGTCAACAACAAAGACCTGCCAA GAATTGATGATGATGGTGAGCAAGAAAACATGCITGAACGACAACAAGATCITGAGAGCCAGTGCGTCTCGTGCACGTCAACAACAAAGACCTGCCAA	2787 2799
crgA(-) crgA(+)	TTCATTATCTACTGCACCTGCATCTCCGTTCGACCAATGACCACTACTACGAATACTACAATGACTCAACCCGCTAGTATGATGGCCAGACCCGCT TTCATTATCTACTGCACCTGCATCTCCTTCCGTTCGACCAATGACCACTACTACGAATACTACAATGACTCAACCCGCTAGTATGATGGCCAGACCCGCT	2887 2899
crgA(-) crgA(+)	AGTATGATCGCTAGACCCGCCAGCATGATGGCCAGACCCGCCAGTATGATCGCTAGACCCGCCAGTATGGCATCCAGATCTAACCCAGCCGTTCGCGCAC AGTATGATCGCTAGACCCGCCAGCATGATGGCCAGACCCGCCAGTATGATCGCTAGACCCGCCAGTATGGCATCCAGATCTAACCCAGCCGTTCGCGAC	2987 2999
crgA(-) crgA(+)	COATGGGTCGCCCAATGCCCCATCAAGTTAGACCTCAAGCTACAAATGCTAGCATGGAACCCAACAATGCTCGTCAATCCTGGGCTCAACGTGCTCATCC CCATGGGTCGCCCAATGCCCCATCAAGTTAGACCTCAAGCTACAAATGCTAGCAAGCA	3087 3099
crgA(-) crgA(+)	TCAGACACAAGCACCTGTTAGTCGAGCTCCTTGGCTTCAAATGCATGTTCAAGGTCTAATGCTCAACGATCTAAGCCTCAATTACAACAACAACAACAA TCAGACACAAAGCACCTGTTAGTCGAGCTCCTTGGGTTCAAATGCATGTTCAAGGTCTAATGCTCAACGATCTAAGCCTCAATTACAACAACAACAA	3187 3199
crgA(-) crgA(+)	CAGCAGCAGCAACAACAGCAATCTCACAACATCCCTATTATACCCGAAAAAACAGTCAAGAATCGTCAAGAACAGACGACTGAAGAAGAGTGATTGAT	3287 3299
crgA(-) crgA(+)	${\tt taacagcttttottgaaaaattattatgtcataagaatgctaatcctaatgacagcatgtctacttggctaagtgccttgggtgatcctcctacattacgtacg$	3387 3399
crgA(-) crgA(+)	${\tt tgetcetcarcgcgatcoretcatcttgacttgetcattarcatgatgeccttgagtgaagatgaaaaggttcatctgataggttcattgatagcatgcat$	3487 3499
crgA(-) crgA(+)	cotoaacccctttgotoatcatttcccctattoatccattcgacactcattcgtctatttaaccaactcatcgtcttactcatcctcatcctcatcctcatcctcatcctcatcctcatcctttaaccaactcatccttcatcctctcatca	3587 3599
crgA(-) crgA(+)	$\tt harcacctgttacttgttgcatttcttataggcccattcctttacttaattattatgccctcactctcatctctctc$	3687 3699
crgA(-) crgA(+)	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	3787 3799
crgA(-) crgA(+)	${\tt rattcagttracattggttratccatcttttgccaataaaaataaatcatgtagtagtagaccactttacctagtgctcttggaagaggagtagggagga$	3887 3899
crgA(-) crgA(+)	CAGTATCACTCCTCAATTGCTGCAATATTTTTGATAAGAACATATGTCTTGGGRAATGTAAAGCCAAAGGAGGGGGAAGAAACCATAAGAGGGAAAGCATA CAGTATCACTCCTCAATTGCTGCAATATTTTTGATAAGAACATATGTCTTGGGAAATGTAAAGCCAAAGGAGGGGAAGAAACCATAAGAGGAAAGCTAA	3987 3999
crgA(-) crgA(+)	${\tt tacgaataaagatcttgtaattccactattaaaaggagattgttcttttcttttttatctacatttgtctttttttt$	4087
crgA(-) crgA(+)	atttatgtanaragggtanagaggganganarararagaggggganggagagagaga	4184 4199
crgA(-) crgA(+)	AAGATCGAACAATTGCTCAAACAATCTCCCCAGTATAGACAAATCTTGTTCCGTATGAAAAT AAGATCGAACAATTGCTCAAAAATCTCCCCAGTATAGACAAATCTTGTTCCATATGAAAAT	4245 4260

Fig.S2A

RF1	
CrgA(-) MQSIQQHKRSRDQVDTLPVGQFNKKQKQIDVFSPSVVVEAFTRCPSCHGK CrgA(+) MQSIQQHKRSRDQVDTLPVGQFNKKQKQIDVFSPSVVVEAFTRCPSCHGK	50 50
CrgA(-)LNKPTTLPCGFTACHACVASSQQCISPTCDRLHTIALQPSVTIRASQAIVCrgA(+)LNKPTTLPCGFTACHACVASSQQCISPTCDRLHTIALQPSVTIQALQAIV	100 100
RF1 RF2	
CrgA(-) VSAEASRTLDTLRLTLDSSTECPICCSRFNNPTTTPCGHTFCRNCLIRSL CrgA(+) VSAEASRTLDTLRLTLDSSTECPICCSRFNNPTTTPCGHTFCRNCLIRSL	150 150
RF2 RF2	
CrgA(-)DHQRSCPFCRDNLDFCPPPAKILCDILSQLYADDAEADEDALAMLDQDVRCrgA(+)DHQRSCPFCRDNLDFCPPPAKILCDILSQLYADDAEADEDALAMLDQDVR	200 200
RF2 LON	
CrgA(-) VPLLIGNLAFPHVKCAIHVFEPRYRLMLRRIMQSNRRRFAMCIARRNRSE CrgA(+) VPLLIGNLAFPHVKCAIHVFEPRYRLMLRRIMQSNRRFAMCIARRNRSE	250 250
CrgA(-) GQAPFYEYGTMLELTHVQTLPDGRSLVEAIGSHRFKVLDYELTDGYHMAS CrgA(+) GQAPFYEYGTMLELTHVQTLPDGRSLVEAIGSHRFKVLDYELTDGYHMAS	300
CrgA(-) IERIDDIDGEQENMLERQQILRASASRARQQQRPANSLSTAPASPSVRPM	350
CrgA(+) IERIDDIDGEQENMLERQQILRASASRARQQQRPANSLSTAPASPSVRPM	350
НР	
CrgA (-) TTTTNTTMTQPASMMARPASMIARPASMIARPASMIARPASMASRSNPAV	400
CrgA(+) TTTTNTTMTQPASMMARPASMIARPASMARPASMARPASMASRSNPAV	400
CrgA(-) RAPMGRPMPHQVRPQATNASMEPNNARQSWAQRAHPQTQAPVSRAPWLQM	450
CrgA(+) RAPMGRPMPHQVRPQATNASMEPNNARQSWAQRAHPQTQAPVSRAPWLQM	450
CrgA(-) HVOGLSAORSKPOLOOOOOOOOOOOOSHNIPIIPEKTVKNROEOTTEEMI	500
CrgA(+) HVQGLSAQRSKPQLQQQQQQQQQQQSHNIPIVPEKTVKNRQEQTTEEMI	500
LON	
CrgA(-) DELTAFVEKLLCHKNANPNDSMSTWLSALGDPPTLRGPQRDRVILTWWVI	550
CrgA(+) DELTAFVEKLLCHKNANPNDSMSTWLSALGDPPTLRGPQRDRVILTWWVI	550
CrgA(-) NMMPLSEDEKVSLIAMRTLRERVLVIISRIDRFESQWSVFLNNSSSTYSS	600
CrgA(+) NMMPLSEDEKVSLIAMRTLRERVLVIISRIDRFESQWSVFLNNSSSTHSS	600
<u>IP</u>	
CrgA(-) SNQTPVTCCIS	611
	011

Fig.S2B





Fig.S3