Fig S1 : sequence alignment of RrgA, RrgB, RrgC, GBS52 CnaB domains. The bars represent the β-strands of the RrgA-D4 (Izoré et al. 2010) and the GBS52-N2 (Krishnan et al. 2007) domains. Residues involved in isopeptide bond formation are indicated in red.

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RrgA-D4       731 -KEKKLGDIEFIKVNKN-DKKP-LRGAVFSLQKQHP------------------------ 763
RrgB-CnaB     441 EPKVVTYGKKFVKVN---DKDNRLAGAEFVIANADNAGQYLARKADKVSQEEKQLVVTTK 497
RrgC-CnaB1    143 KTDTMTTKVKLIKVDQD---HNRLEGVGFKLVSVAR------------------------ 175
RrgC-CnaB2    253 -QKLPRGNVDFMKVDGR--TNTSLQGAMFKVMKEES------------------------ 285
Gbs52-N2      151 ---WSTGELDLLKVGVDGDTKKPLAGVVFELYEKNG------------------------ 183


RrgA-D4       764 ----------------------------------------DYPDIYGAIDQNGTYQNVRTG 784
RrgB-CnaB     498 DALDRAVAAYNALTAQQQTQQEKEKVDKAQAAYNAAVIAANNAFEWVADKDNENVVKLVSD 558
RrgC-CnaB1    176 ---------------------------------DVSEKEVPLIGEYRYSSSGQVGRTLYTD 203
RrgC-CnaB2    286 -------------------------------------------GHYTPVLQNGKEVVVTSG 303
Gbs52-N2      184 -------------------------------------RTPIRVKNGVHSQDIDAAKHLETD 207


RrgA-D4       785 EDGKLTFKNLSDGKYRLFENSEPAGYKPVQNKPIVAFQIV----NGEVRDVTSIVPQDI 839
RrgB-CnaB     559 AQGRFEITGLLAGTYYLEETKQPAGYALLTSRQKFEVTATSYSATGQGIEYTAGSGKDD 617
RrgC-CnaB1    204 KNGEIFVTNLPLGNYRFKEVEPLAGYAVTTLDTDVQLVDHQLVTITVVNQKLPRGNVDF 262
RrgC-CnaB2    304 KDGRFRVEGLEYGTYYLWELQAPTGYVQLTSPVSFTIGKDTRKELVTVVKNNKRPRIDV 362
Gbs52-N2      208 SSGHIRISGLIHGDYVLKEIETQSGYQIGQAETAVTIEKSKTVTVTIENKKVPTPKVPS 266


RrgA-D4       840 PAGYEFTNDKHYITNEPIPPK 860
RrgB-CnaB     618 ATKVVNKK------------- 625
RrgC-CnaB1    263 MKVDGRTNT------------ 271
RrgC-CnaB2    363 PDTGEETLYI----------- 372
Gbs52-N2      267 RGGLIPKTG------------ 275
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Fig S2 : Structural superposition of RrgA-D4 (orange) and GBS52-N2 (gray) CnaB-domains. For figure clarity, the loops have been removed. The intramolecular bonds and the glutamate residues are represented by sticks, RrgA (red) and GBS52 (blue).

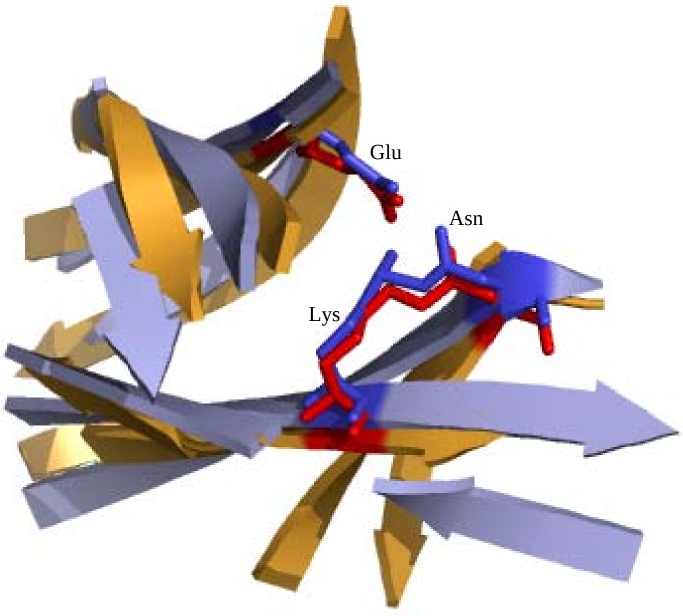

Table S1. Primers used for the co-expression constructs.

Primer	Sequence
RrgB_F63Eco	5' CGC G ATA TCG GCT GGG ACG ACA ACA ACA TCT GTT ACC 3’
RrgB_R63Xho	5’ GCG CTC GAG ACC ACC CGT TTG TGG GAT AGT G 3’
RrgC_F64Bam	5' CGC G GAT CCG GTC CAA GCG CAA GAA GAT CAC ACG 3'
RrgC_R64Hind	5’ GCG AAG CTT TCA TTC TTC CCC TGT ATC TGG CAC 5’
SrtC1_F66Nde	5' CGC CAT ATG GAA TCA AAT CAA CAA ATT GCT GAC 3’
SrtC1_R66Xho	5’ GCG CTC GAG TCA ATG ACT GAG TTT GTT TGC TGC AAT AAA 3’

Table S2. Strains used in the co-expression experiments.

Plasmids transformed into *E.coli Star* strains	Proteins co-expressed
pLIM01 RrgB	H6-RrgB$_{30\text{-}633}$
pETDuet RrgB	RrgB$_{30\text{-}633}$-S-Tag
pACYCDuet RrgC	H6-RrgC$_{22\text{-}368}$
pACYCDuet SrtC-1	SrtC-1$_{17\text{-}228}$
pETDuet RrgA + pACYCDuet SrtC-1	H6-RrgA$_{39\text{-}868}$; SrtC-1$_{17\text{-}228}$
pETDuet RrgB + pACYCDuet SrtC-1	RrgB$_{30\text{-}633}$-S-Tag ; SrtC-1$_{17\text{-}228}$
pACYCDuet RrgC_SrtC1	H6-RrgC$_{22\text{-}368}$; SrtC-1
pETDuet RrgA_RrgB + pACYCDuet SrtC-1	H6-RrgA$_{39\text{-}868}$; RrgB$_{30\text{-}633}$-S-Tag ; SrtC-1$_{17\text{-}228}$
pETDuet RrgA + pACYCDuet RrgC_SrtC1	H6-RrgA$_{39\text{-}868}$; H6-RrgC$_{22\text{-}368}$; SrtC-1$_{17\text{-}228}$
pETDuet RrgB + pACYCDuet RrgC_SrtC1	RrgB$_{30\text{-}633}$-S-Tag ; H6-RrgC$_{22\text{-}368}$; SrtC-1$_{17\text{-}228}$
pETDuet RrgA_RrgB + pACYCDuet RrgC_SrtC1	H6-RrgA$_{39\text{-}868}$;RrgB$_{30\text{-}633}$-S-Tag ; H6-RrgC$_{22\text{-}368}$; SrtC-1$_{17\text{-}228}$

Table S3. Summary of the peptides identified from the RrgB-RrgC complex by MS/MS.

MS/MS analysis of the RrgB-RrgC complex

Observed	Mr (expt)	Mr (calc)	Delta	Start	Stop	Sequence	Identified protein
521,75	1041,48	1041,48	0,00	243	251	MTEGLAFNK	RrgB
523,79	1045,56	1045,56	0,00	129	138	FNTANLPAAK	RrgB
555,75	1109,49	1109,49	0,00	42	51	LLATDGDMDK	RrgB
556,79	1111,56	1111,56	0,00	199	208	ANPDTPRVDK	RrgB
563,29	1124,57	1124,57	0,00	300	309	ITYSATLNDK	RrgB
571,27	1140,52	1140,52	0,00	109	119	LSGAMPATAMK	RrgB
588,82	1175,63	1175,63	0,00	580	590	QPAGYALLTSR	RrgB
602,84	1203,67	1203,67	0,00	377	387	VVQTVTLTTDK	RrgB
635,31	1268,61	1268,61	0,00	109	120	LSGAMPATAMKK	RrgB
679,89	1357,76	1357,76	0,00	491	502	QLVVTTKDALDR	RrgB
714,36	1426,71	1426,71	0,00	243	255	MTEGLAFNKGTVK	RrgB
740,37	1478,73	1478,73	0,01	431	443	DENPKPLDPTEPK	RrgB
744,92	1487,82	1487,82	0,00	485	497	VSQEEKQLVVTTK	RrgB
747,37	1492,72	1492,72	0,00	52	65	IANELETGNYAGNK	RrgB
831,92	1661,82	1661,82	0,00	228	242	IPALANYATANWSDR	RrgB
843,94	1685,86	1685,86	0,01	281	296	LTDAGLAKVNDQNAEK	RrgB
923,49	1844,97	1844,96	0,00	121	138	LTEAEGAKFNTANLPAAK	RrgB
958,47	1914,93	1914,92	0,00	410	427	GYSADYQEITTAGEIAVK	RrgB
644,37	1930,08	1930,07	0,00	481	497	KADKVSQEEKQLVVTTK	RrgB
982,00	1961,98	1961,98	0,00	503	520	AVAAYNALTAQQQTQQEK	RrgB
987,54	1973,06	1973,06	0,00	120	138	KLTEAEGAKFNTANLPAAK	RrgB
687,04	2058,10	2058,10	0,00	485	502	VSQEEKQLVVTTKDALDR	RrgB
1032,53	2063,05	2063,04	0,01	461	480	LAGAEFVIANADNAGQYLAR	RrgB
1071,54	2141,07	2141,06	0,00	209	227	DTPVNHQVGDVVEYEIVTK	RrgB
1110,57	2219,12	2219,12	0,00	503	522	AVAAYNALTAQQQTQQEKEK	RrgB
1166,14	2330,26	2330,25	0,01	163	183	AVPIEIELPLNDVVDAHVYPK	RrgB
1171,09	2340,16	2340,15	0,01	141	162	IYEIHSLSTYVGEDGATLTGSK	RrgB
781,75	2342,23	2342,22	0,01	52	74	IANELETGNYAGNKVGVLPANAK	RrgB
828,76	2483,26	2483,25	0,00	206	227	VDKDTPVNHQVGDVVEYEIVTK	RrgB
845,10	2532,27	2532,26	0,01	498	520	DALDRAVAAYNALTAQQQTQQEK	RrgB
854,78	2561,31	2561,31	0,00	503	525	AVAAYNALTAQQQTQQEKEKVDK	RrgB
861,09	2580,24	2580,23	0,01	591	615	QKFEVTATSYSATGQGIEYTAGSGK	RrgB
862,41	2584,20	2584,20	0,00	42	65	LLATDGDMDKIANELETGNYAGNK	RrgB
1306,66	2611,30	2611,29	0,01	256	280	VTVDDVALEAGDYALTEVATGFDLK	RrgB
1316,66	2631,31	2631,31	0,01	139	162	YKIYEIHSLSTYVGEDGATLTGSK	RrgB
1037,83	3110,48	3110,47	0,01	591	620	QKFEVTATSYSATGQGIEYTAGSGKDDATK	RrgB
1079,22	3234,62	3234,62	0,01	199	227	ANPDTPRVDKDTPVNHQVGDVVEYEIVTK	RrgB
1127,92	3380,74	3380,72	0,02	256	288	VTVDDVALEAGDYALTEVATGFDLKLTDAGLAK	RrgB
1145,58	3433,71	3433,70	0,01	42	74	LLATDGDMDKIANELETGNYAGNKVGVLPANAK	RrgB
983,77	3931,05	3931,04	0,01	555	590	LVSDAQGRFEITGLLAGTYYLEETKQPAGYALLTSR	RrgB

Observed	Mr (expt)	Mr (calc)	Delta	Start	Stop	Sequence	Identified protein
470,73	939,44	939,44	0,00	190	198	YSSSGQVGR	RrgC
538,29	1074,57	1074,57	0,00	181	189	EVPLIGEYR	RrgC
572,26	1142,50	1142,50	0,00	72	80	DLHSWDENK	RrgC
573,81	1145,61	1145,60	0,00	297	307	EVVVTSGKDGR	RrgC
607,30	1212,58	1212,58	0,00	269	279	TNTSLQGAMFK	RrgC
624,75	1247,50	1247,49	0,00	57	66	LDDSYSYDDR	RrgC
779,88	1557,75	1557,74	0,00	283	296	EESGHYTPVLQNGK	RrgC
817,43	1632,84	1632,84	0,00	176	189	DVSEKEVPLIGEYR	RrgC
853,42	1704,82	1704,81	0,01	72	85	DLHSWDENKLSSFK	RrgC
853,95	1705,88	1705,88	0,00	205	219	NGEIFVTNLPLGNYR	RrgC
611,98	1832,91	1832,91	0,00	72	86	DLHSWDENKLSSFKK	RrgC
615,30	1842,88	1842,87	0,00	57	71	LDDSYSYDDRVQIVR	RrgC
974,98	1947,94	1947,94	0,00	280	296	VMKEESGHYTPVLQNGK	RrgC
786,74	2357,19	2357,19	0,00	283	304	EESGHYTPVLQNGKEVVVTSGK	RrgC
1214,63	2427,25	2427,24	0,01	199	219	TLYTDKNGEIFVTNLPLGNYR	RrgC
896,12	2685,34	2685,34	0,00	283	307	EESGHYTPVLQNGKEVVVTSGKDGR	RrgC
916,80	2747,38	2747,38	0,00	280	304	VMKEESGHYTPVLQNGKEVVVTSGK	RrgC
755,62	3018,45	3018,44	0,01	87	111	TSFEMTFLENQIEVSHIPNGLYYVR	RrgC
1026,19	3075,53	3075,53	0,01	280	307	VMKEESGHYTPVLQNGKEVVVTSGKDGR	RrgC