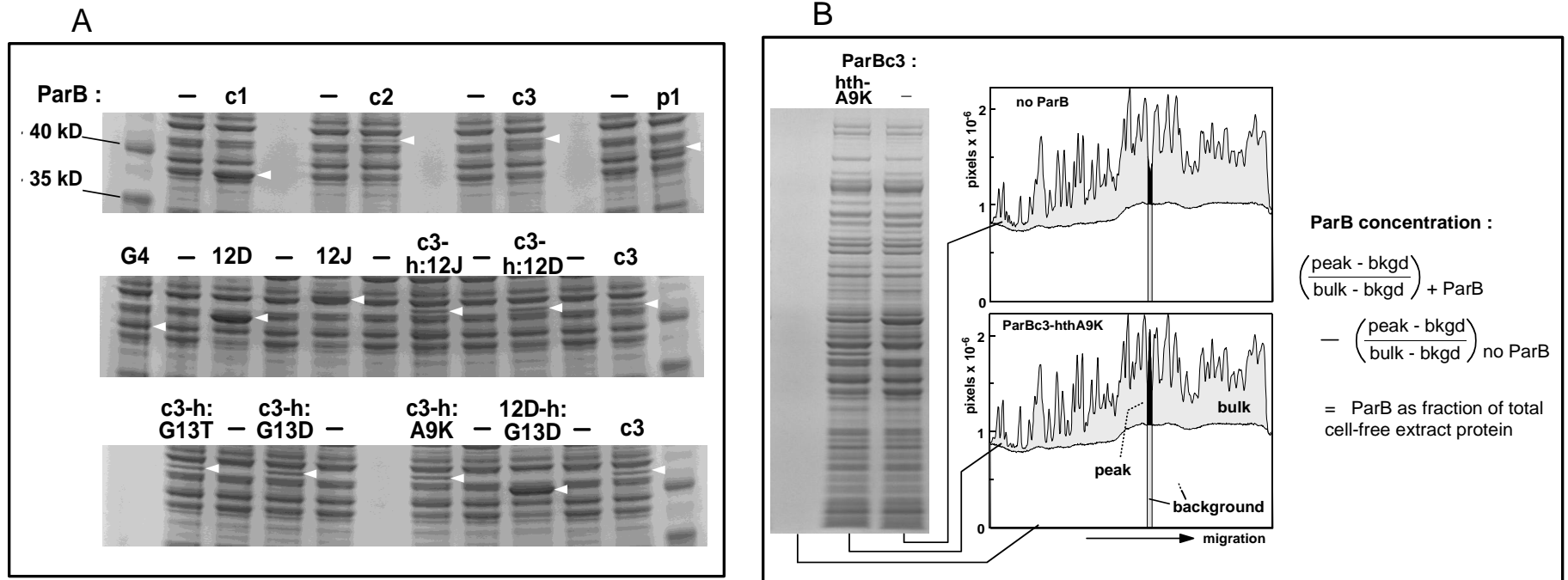
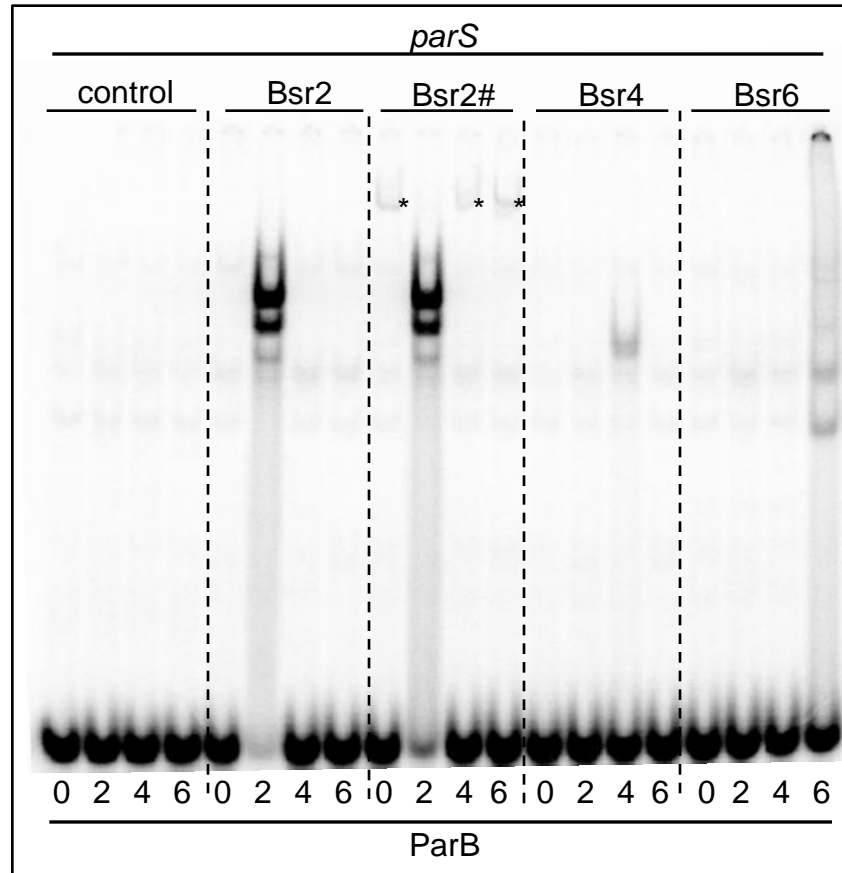


Figure S1. Estimation of ParB concentration in cell-free extracts



A. Electrophoretic fractionation of extract proteins in denaturing polyacrylamide gels; only the segments including ParB polypeptides (arrowheads) are shown. Lanes containing no protein (omitted from the example shown in the second panel) or protein from cells without ParB were included to enable quantitation. Stained gel images were captured with a gel-imager (Syngene G box) as .tif files and processed using Multi Gauge (Fuji). Size standards are the prestained PageRuler proteins from Fermentas. All ParB polypeptides migrated as species 5-10 kD larger than these markers; abnormal migration of ParB family proteins has been observed previously (e.g. Phua, S. H., P. L. Bergquist, and H. E. Lane. 1982. Effects of Tn5 insertion in the incD region on mini-F maintenance and polypeptide synthesis. Mol. Gen. Genet. 188:353–355.). **B.** Method for estimating ParB concentrations. The example of ParBc3-hthA9K, including empty and non-ParB lanes, is shown. Total protein was estimated by subtracting the entire background (empty lane) scan from that of the protein-containing lanes, and ParB by applying the same process to the defined peak area. The difference of the two ParB/total protein fractions gives ParB as a proportion of the 3.5 µg of protein loaded in each lane. Obviously, such a procedure can only yield approximate values, but cases where replicate estimates were made (Table S1; *B.cen* c3, *R.Pic* 12D c1) indicate that it is accurate within a factor of about 2.5.

Figure S2. EMSA with ParB proteins and *parS* sequences from Bsr2, Bsr4 and Bsr6



The ParB protein overexpressed in the extract is indicated below each lane as follows: 0 - no ParB; 2 – Bsr2-ParB from *B.cenocepacia* chromosome 2; 4 – Bsr4-ParB from the Mega-plasmid of *Ralstonia solanacearum* GM11000; 6 – Bsr6-ParB, from the plasmid 2 of *Polaromonas naphthalenivorans* CJ2 (pNAP2). The *parS* probes are indicated above each panel and have the following internal sequences: control – ctagtcgtacgactag; Bsr2 – gtttatgcgcataaac; Bsr2# (a secondary site of *B.cen c2*) gtttatgcgcacaaac; Bsr4 – ctttcagcgctgaaag; Bsr6 - tgttctgcgcagaaca. The asterisk indicates a complex formed by an *E. coli* protein with *parS*-Bsr2#.

TABLE S1. ParB concentrations in cell-free extracts

ParB	monomer mol. wt. (kD)	% total protein	concentration in extract (µg/ml)
<i>B. cenocepacia</i> J2315 c1	33.0	3.7	2.8
<i>B. cenocepacia</i> J2315 c2	39.6	1.6	1.1
<i>B. cenocepacia</i> J2315 c3	37.5	1.5, 1.0, 1.9	1.1
<i>B. cenocepacia</i> J2315 pBC	34.9	0.6	0.4
<i>B. vietnamiensis</i> G4 p2	32.0	0.9	0.6
<i>R. pickettii</i> 12D p1	32.8	2.6	1.9
<i>R. pickettii</i> 12J c1::p	32.9	2.4	2.0
<i>B. cenocepacia</i> c3-hth12J ^a	37.5	1.1	0.8
<i>B. cenocepacia</i> c3-hth12D ^a	37.5	0.9	0.6
<i>B. cenocepacia</i> c3-hth.G13T ^a	37.5	1.3	0.9
<i>B. cenocepacia</i> c3-hth.G13D ^a	37.5	2.3	1.6
<i>B. cenocepacia</i> c3-hth.A9K ^a	37.5	2.3	1.8
<i>R. pickettii</i> 12D p1-hth.D13G ^a	32.8	5.1	4.0

C1, c2 and c3 refer to chromosome 1, chromosome 2 and chromosome 3 of the indicated species. pBC refers to the plasmid of *B. cenocepacia* J2315. P1 and p2 refer to plasmids 1 and 2 of the indicated species. C1::p refers to the plasmid integrated in c1 of *R. pickettii* 12J. ^a proteins modified as indicated in Fig. 6

TABLE S2. ParB proteins used for the phylogenetic analysis

BsrType	Strain	Prot. Acces.N°	Locus tag	Uniprot	Genome ^a
RepB	<i>Agrobacterium tumefaciens</i>	YP_001967488.1	pTiBo087	A5WY07	pTiBo542
RepB	<i>Rhizobium leguminosarum</i> viciae 3841	YP_770903.1	pRL80002	Q1M9K4	pRL8
RepB	<i>Agrobacterium vitis</i> S4	YP_002542550.1	Avi_9002	B9K3B7	pAtS4c
RepB	<i>Nitrobacter hamburgensis</i> X14	YP_571717.1	Nham_4280	Q1QFW2	pNITHX1
RepB	<i>Sinorhizobium meliloti</i> BL225C	ZP_07581171.1	SinmeBDRAFT_1526	E0JCB8	-
RepB	<i>Rhizobium etli</i> IE4771	ZP_03514520.1	RetI_010100002436	-	-
RepB	<i>Methylobacterium nodulans</i> ORS 2060	YP_002498945.1	Mnod_3735	B8IRA2	-
RepB	<i>Mesorhizobium</i> sp. BNC1	YP_665972.1	Meso_4355	Q11MP4	pBNC1-1
RepB	<i>Bradyrhizobium</i> sp. BTAi1	YP_001220373.1	BBta_p0002	A5EU57	pBBta01
chr1	<i>Burkholderia ambifaria</i> IOP40-10	ZP_02890508	BamIOP4010DRAFT-2571	B1FEW1	-
chr1	<i>Burkholderia ambifaria</i> MC40-6	YP_001806815	BamMC406_0097	B1YQK5	chr1
chr1	<i>Burkholderia ambifaria</i> AMMD	ABI85648	Bamb_0087	Q0BJM5	chr1
chr1	<i>Burkholderia ambifaria</i> MEX-5	ZP_02905066	BamMEX5DRAFT_0420	B1SY04	-
chr1	<i>Burkholderia cenocepacia</i> AU 1054	ABF77855	Bcen_2959	Q1BRA0	chr1
chr1	<i>Burkholderia cenocepacia</i> MC0-3	ACA89293	Bcenmc03_0113	B1JSU8	chr1
chr1	<i>Burkholderia cenocepacia</i> HI2424	ABK06850	Bcen2424_0096	A0K2X3	chr1
chr1	<i>Burkholderia cenocepacia</i> PC184	ZP_04940010	BCPG_01446	A2VTY5	-
chr1	<i>Burkholderia</i> sp. 383	ABB06880	Bcep18194_A3278	Q39KY6	chr1
chr1	<i>Burkholderia vietnamiensis</i> G4	ABO53128	Bcep1808_0105	A4JA25	chr1
chr1	<i>Burkholderia ubonensis</i> Bu	ZP_02380326	BuboB_010100021506	-	-
chr1	<i>Burkholderia multivorans</i> CGD1	ZP_03587270	BURMUCGD1_0048	B9BCF5	-
chr1	<i>Burkholderia multivorans</i> CGD2	ZP_03581750	BURMUCGD2_0046	B9BZG0	-
chr1	<i>Burkholderia multivorans</i> ATCC 17616	BAG45043	BMULJ_03169	A9AJF4	chr1
chr1	<i>Burkholderia dolosa</i> AUO158	ZP_04944299	BDAG_00147	A2W674	-
chr1	<i>Burkholderia glumae</i> BGR1	YP_002909975	bglu_1g00680	C5A862	chr1
chr1	<i>Burkholderia xenovorans</i> LB400	ABE32896	Bxe_A0031	Q13SP3	chr1
chr1	<i>Burkholderia graminis</i> C4D1M	ZP_02885466	BgramDRAFT_4276	B1G4P2	-
chr1	<i>Burkholderia phytofirmans</i> PsJN	ACD18289	Bphyt_3902	B2T7K8	chr1
chr1	<i>Burkholderia phymatum</i> STM815	YP_001859252	Bphy_3036	B2JJK8	chr1
chr1	<i>Ralstonia solanacearum</i> GMI1000	NP_521444	RSc3325	Q8XU68	chr1
chr1	<i>Ralstonia pickettii</i> 12D	YP_002983134	Rpic12D_3195	C6BH88	chr1
chr1	<i>Ralstonia pickettii</i> 12J	YP_001901072	Rpic_3520	B2UGV7	chr1
chr1	<i>Polaromonas naphthalenivorans</i> CJ2	ABM35394	Pnap_0068	A1VIB6	chr1
chr1	<i>Polaromonas</i> sp. JS666	ABE42045	Bpro_0078	Q12HE7	chr1
chr1	<i>Rhodiferax ferrireducens</i> DSM 15236	ABD67815	Rfer_0053	Q223L5	chr1
chr1	<i>Acidovorax ebreus</i> TPSY	YP_002551539	Dtpsy_0050	B9M9W8	chr1
Bsr1_a	<i>Burkholderia cenocepacia</i> HI2424	ABK13603	Bcen2424_6874	A0B4V9	plasmid1
Bsr1_a	<i>Burkholderia cenocepacia</i> J2315	YP_002235439	pBCA002	B4EQN6	pBCJ2315
Bsr1_a	<i>Burkholderia glumae</i> BGR1	YP_002909815	bglu1p0020	C5AMY6	bglu_1p
Bsr1_a	<i>Burkholderia ambifaria</i> IOP40-10	ZP_02888520	BamIOP4010DRAFT_0582	B1F973	-
Bsr1_a	<i>Burkholderia multivorans</i> ATCC 17616	YP_001573704	Bmul_6251	A9AU38	pBMUL01
Bsr1_a	<i>Burkholderia multivorans</i> ATCC 17616	BAG48097	BMULJ_06311	A9AU38	pTGL1
Bsr1_b	<i>Polaromonas naphthalenivorans</i> CJ2	YP_973272	Pnap_4247	A1VV52	pPNAP01
Bsr2	<i>Burkholderia xenovorans</i> LB400	ABE32970	Bxe_B3027	Q13SG9	chr2
Bsr2	<i>Burkholderia phytofirmans</i> PsJN	ACD21298	Bphyt_7010	B2T7Y2	chr2
Bsr2	<i>Burkholderia graminis</i> C4D1M	ZP_02882092	BgramDRAFT_0901	B1FUM7	-
Bsr2	<i>Burkholderia phymatum</i> STM815	YP_001860319	Bphy_4152	B2JPT2	chr2
Bsr2	<i>Burkholderia mallei</i> NCTC 10229	ABM98834	BMA10229_1423	-	chr2
Bsr2	<i>Burkholderia pseudomallei</i> K96243	CAH39837	BPSS2350	Q63HR9	chr2
Bsr2	<i>Burkholderia pseudomallei</i> 1106a	ABN95486	BURPS1106A_A3177	A3PA46	chr2
Bsr2	<i>Burkholderia mallei</i> JHU	ZP_04915784	BMAJHUJ_0006	-	-

Bsr2	Burkholderia glumae BGR1	YP_002907735	bglu_2g00020	C5AKP4	chr2
Bsr2	Burkholderia ubonensis Bu	ZP_02376085	BuboB_010100000065	-	-
Bsr2	Burkholderia multivorans ATCC 17616	BAG47198	BMULJ_05361	A9AKT1	chr2
Bsr2	Burkholderia multivorans CGD1	ZP_03583495	BURMUCGD1_5586	B9B0W6	-
Bsr2	Burkholderia multivorans CGD2	ZP_03577302	BURMUCGD2_6020	B9BLR2	-
Bsr2	Burkholderia dolosa AUO158	ZP_04948154	BDAG_04158	A2WH79	-
Bsr2	Burkholderia vietnamiensis G4	ABO56777	Bcep1808_3794	A4JKH4	chr2
Bsr2	Burkholderia cepacia AMMD	ABI90515	Bamb_4966	Q0B5Q8	chr2
Bsr2	Burkholderia ambifaria MC40-6	YP_001809798	BamMC406_3107	B1YXN2	chr2
Bsr2	Burkholderia ambifaria IOP40-10	ZP_02890407	BamIOP4010DRAFT_2470	B1FEL0	-
Bsr2	Burkholderia ambifaria MEX-5	ZP_02906429	BamMEX5DRAFT_1783	B1T1W7	-
Bsr2	Burkholderia sp. 383	ABB10121	Bcep18194_B0004	Q39BP0	chr2
Bsr2	Burkholderia cenocepacia MC0-3	ACA93682.1	Bcenmc03_4542	B1K276	chr2
Bsr2	Burkholderia cenocepacia HI2424	ABK12427.1	Bcen2424_5694	A0B451	chr2
Bsr2	Burkholderia cenocepacia AU 1054	ABF80039.1	Bcen_5165	Q1BK16	chr2
Bsr2	Burkholderia cenocepacia PC184	ZP_04942860	BPCPG_04404	A2W235	-
Bsr2	Burkholderia cenocepacia J2315	YP_002232636	BCAM0004	B4EFY9	chr2
Bsr3_a	Burkholderia multivorans ATCC 17616	BAG47206	BMULJ_05369	A9ATE3	chr3
Bsr3_a	Burkholderia multivorans CGD1	ZP_03585227	BURMUCGD1_6471	B9B6L2	-
Bsr3_a	Burkholderia multivorans CGD2	ZP_03578401	BURMUCGD2_6710	B9BPT9	-
Bsr3_a	Burkholderia cenocepacia HI2424	ABK12926.1	Bcen2424_6194	A0KCL3	chr3
Bsr3_a	Burkholderia cenocepacia AU 1054	ABF80692.1	Bcen_5827	Q1BI63	chr3
Bsr3_a	Burkholderia cenocepacia MC0-3	ACA95785.1	Bcenmc03_6671	B1KC38	chr3
Bsr3_a	Burkholderia cenocepacia PC184	ZP_04943653	BPCPG_05224	A2W4C8	-
Bsr3_a	Burkholderia cenocepacia J2315	YP_002153395	BCAS0002	B4EQD0	chr3
Bsr3_a	Burkholderia sp. 383	ABB06782.1	Bcep18194_C7738	Q39L84	chr3
Bsr3_a	Burkholderia vietnamiensis G4	ABO58426.1	Bcep1808_5482	A4JQ73	chr3
Bsr3_a	Burkholderia cepacia AMMD	ABI91545.1	Bamb_6001	Q0B2S8	chr3
Bsr3_a	Burkholderia ambifaria IOP40-10	ZP_02891804	BamIOP4010DRAFT_3867	B1FIK7	-
Bsr3_a	Burkholderia ambifaria MC40-6	YP_001815752	BamMC406_5757	B1Z603	chr3
Bsr3_a	Burkholderia ambifaria MEX-5	ZP_02908427	BamMEX5DRAFT_3781	B1T7L5	-
Bsr3_a	Burkholderia dolosa AUO158	EAY71634	BDAG_04474	A2WI38	-
Bsr3_a	Burkholderia ubonensis Bu	ZP_02381864	BuboB_010100029348	-	-
Bsr3_b	Ralstonia pickettii 12D	YP_002973646	Rpic12D_5174	C6BQP4	pRp12D02
Bsr3_b	Ralstonia pickettii 12J	YP_001899080	Rpic_1509	B2UCJ6	chr1
Bsr3_c	Ralstonia pickettii 12D	YP_002979969	Rpic12D_5081	C6BQ41	pRp12D01
Bsr3_d	Burkholderia vietnamiensis G4	YP_001109717	Bcep1808_7053	A4JU13	pBVIE02
Bsr4	Ralstonia pickettii 12D	YP_002983826	Rpic12D_3897	C6BM46	chr2
Bsr4	Ralstonia pickettii 12J	YP_001892327	Rpic_3784	B2UH49	chr2
Bsr4	Ralstonia solanacearum GMI1000	NP_521565	RSp0004	Q8XTV0	megaplasmid
Bsr4	Cupriavidus metallidurans CH34	YP_587948	Rmet5820	Q1LAZ7	megaplasmid
Bsr4	Cupriavidus pinatubonensis	AAZ64689.1	Reut_B5344	Q46Q95	chr2
Bsr4	Cupriavidus necator	CAJ94810.1	H16_B0004	Q0K5B4	chr2
Bsr4	Cupriavidus taiwanensis LMG 19424	YP_001796449	RALTA_B0005	B2AHG1	chr2
Bsr5_a	Polaromonas sp. JS666	YP_551814	Bpro_5044	Q120X6	p1
Bsr5_b	Polaromonas sp. JS666	YP_552265	Bpro_5514	Q11ZC5	p2
Bsr5_c	Rhodoferrax ferrireducens T118	ABD71971	Rfer_4284	Q21QH4	p1
Bsr6	Polaromonas naphthalenivorans CJ2	ABM39747	Pnap_4471	A1VVR9	pPNAP02
Bsr7	Burkholderia ambifaria IOP40-10	ZP_02891570	BamIOP4010DRAFT_3633	B1FHX3	-
Bsr7	Burkholderia ambifaria MEX-5	ZP_02907883	BamMEX5DRAFT_3237	B1T621	-
Bsr7	Burkholderia glumae BGR1	YP_002913204	bglu4p0020	C5ANU1	bglu_4p
Bsr7	Burkholderia multivorans CGD1	ZP_03588332	BURMUCGD1_6609	B9BFG7	-

^a dash - incomplete genomes; chr - chromosome; p - plasmid

