Supplementary Material

Recombination proteins differently control the acquisition of homeologous DNA during natural *Bacillus subtilis* chromosomal transformation

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This supplementary material contains

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Annex 1. Why *Bacillus subtilis* was selected as the experimental system

B. subtilis competent cells selected to analyse how the recombination machinery limits heterogamic chromosomal transformation (CT): first, its uptake apparatus internalises any environmental DNA with similar efficiency [1,2]. Second, the recombination between the incoming ssDNA and the non-replicating haploid genome of a competent cell simplifies the interpretation of the data [1,3]. Finally, these cells show no codon usage preferences [4].

Annex 2. Donor DNAs

In all cases, a 2997-bp fragment of the *rpoB*482 gene was *in vitro* synthesized and cloned into the *E. coli* pUC57 plasmid [5]. The *rpoB*482 mutation is a single C to T transition, that confers Rif^R, but does not affect RNA polymerase activity. For homogamic CT, plasmid pCB980-borne *rpoB*482 of *B. subtilis* 168 (*Bsu* 168 [1 mismatch]) was used. For heterogamic CT the 2997-bp *rpoB*482 donor DNA was derived from: i) bacteria of the *B. subtilis* clade, with up to ~8% sequence divergence (SD) (pCB981-borne *rpoB*482 of *B. subtilis* W23 (*Bsu* W23 [2.47% SD, 74 mismatches]), pCB982-borne *rpoB*482 of *B. atrophaeus* 1942 (*Bat* 1942, 8.35% SD, 250 mismatches]), ii) a bacterium of the *B. amyloliquefaciens* clade with 10.12% SD (pCB983-borne *rpoB*482 of *B. amyloliquefaciens* DSM7 (*Bam* DSM7, 303 mismatches]); iii) bacteria of the *B. licheniformis* clade: with 14.52% (pCB984-borne *rpoB*482 of *B. licheniformis* DSM13 [*Bli* DSM13, 435 mismatches]) and 17% SD (pCB1054-borne *rpoB*482 of *B. gobiensis* FJAT4402 (*Bgo* FJAT4402, 510 mismatches/insertion/deletion]); iv) a bacterium of the *B. thuringiensis* clade with 20.83% SD (pCB985-borne *rpoB*482 of *B. thuringiensis* MC28 [*Bth* MC28, 624 mismatches/insertion/deletion]); and v) a far distant *Bacillus* with 22.74% SD (pCB1056-borne *rpoB*482 DNA of *B. smithii* DSM4216 [*Bsm* DSM4216, 681 mismatches/insertion/deletion]): All donor *rpoB*482 DNAs have similar dG + dC content. Plasmids were purified and used as donor DNA. This *E. coli* plasmid cannot replicate into *B. subtilis* cells, but the homologous *rpoB*482 DNA integrates and confers Rif^R upon CT.

Previously, it has been shown that expression of a plasmid-borne *B. thuringiensis rpoB*482 gene, which confers Rif^R and express a RpoB protein with 20.83% SD, shows no apparent fitness cost in the recipient strain [5]

Annex 3. RecD2 controls the appearance of spontaneous mutations

The rate of appearance of spontaneous Rif^R mutations was assessed in the different strains listed in Table 1. In the different *rec*$^-$ strains, the number of Rif^R colonies that appeared in the absence of *rpoB*482 DNA was similar to the *wt* control, except in Δ*recD2* cells. The mutation frequencies were similar in the Δ*rok* or *rok*$^+$ backgrounds (~7 to 9 x 10^{-9}). However, the frequency of appearance of Rif^R colonies increased ~3-fold (~2 to 4 x 10^{-8}) in competent Δ*rok* Δ*recD2*, as in Δ*recD2* cells [6]. Similarly, inactivation of *recD*2 increased 3.6-fold the frequency of spontaneous trimethoprim resistant mutants, and the mutation frequency was also increased in *B. anthracis recD2* mutants [7,8]. It becomes clear that RecD2 controls the appearance of spontaneous mutations by an unknown mechanism.

Supplementary references

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Table S1. Mean integration length during interspecies chromosomal transformation in *ΔaddAB, ΔrecO, recF15, ΔruvAB* and *ΔrecU* mutants

Genetic background	Divergence (in %)	Left end (MEPS in bp)	Right end (MEPS in bp)	Integration (in bp)[a]
ΔaddAB	8.35	792 (50)	1647 (17)	855
		780 (11)	1589 (81)	809
		1128 (23)	1867 (41)	739
		1152 (23)	1729 (23)	577
		1407 (36)	1867 (41)	460
	10.12	1128 (23)	1771 (26)	643
		1152 (20)	1771 (26)	619
		1251 (26)	1525 (17)	274
		1356 (14)	1589 (54)	233
		1371 (14)	1589 (54)	218
	14.52	1335 (20)	1588 (11)	253
		1335 (20)	1564 (8)	229
		1335 (20)	1555 (17)	220
		1335 (20)	1522 (14)	187
		1410 (14)	1588 (11)	178
		-	-	(5)[a]
	17.0	1440	1448	~8
		1441	1445	~4
		1441	1445	~4
		1441	1445	~4
		-	-	(3)[a]
ΔrecO	8.35	1251 (18)	2132 (33)	881
		780 (11)	1589 (81)	809
		1128 (23)	1867 (41)	739
		1128 (23)	1729 (23)	601
		1128 (23)	1647 (17)	519
	10.12	1117 (12)	1589 (54)	472
		1300 (17)	1663 (11)	363
		1300 (17)	1589 (54)	289
		1356 (14)	1589 (54)	233
		1371 (14)	1589 (54)	218
	14.52	1309 (11)	1555 (17)	246
		1335 (20)	1555 (17)	220
		1335 (20)	1522 (14)	187
		1410 (14)	1588 (11)	178
		1410 (14)	1555 (17)	145
		-	-	(4)[a]
	17.0	1441	1448	~7
		1441	1445	~4
		1441	1445	~4
		1441	1445	~4
		-	-	(3)[a]
	8.35	1251 (18)	2132 (33)	881
		780 (11)	1589 (81)	809
		792 (50)	1589 (81)	797
		954 (35)	1729 (23)	775
		954 (35)	1589 (81)	635
	10.12	1128 (23)	1771 (26)	643
		1250 (26)	1615 (11)	365
		1251 (26)	1589 (54)	339
		1371 (14)	1663 (11)	292

recF15		1371 (14)	1589 (54)	218
	14.52	1212 (12)	1690 (14)	478
		1251 (14)	1715 (11)	464
		1374 (8)	1690 (14)	316
		1386 (8)	1588 (11)	202
		1335 (20)	1522 (14)	187
		-	-	(5)[a]
	17.0	1441	1448	~7
		1441	1445	~4
		1441	1445	~4
		1441	1445	~4
		-	-	(2)[a]
ΔruvAB	8.35	1342 (11)	1589 (81)	211
		954 (35)	1589 (81)	635
		1128 (23)	1729 (23)	601
		1152 (23)	1729 (23)	577
		1407 (36)	1867 (41)	460
	10.12	1128 (23)	1771 (26)	643
		1150 (20)	1771 (26)	621
		1250 (26)	1525 (17)	275
		1371 (14)	1615 (11)	244
		1371 (14)	1589 (54)	218
	14.52	1335 (20)	1690 (14)	355
		1335 (20)	1588 (11)	253
		1335 (20)	1555 (17)	220
		1335 (20)	1507 (14)	172
		1410 (14)	1555 (17)	145
	17.0	1439	1445	~6
		1441	1448	~7
		1441	1445	~4
		1441	1445	~4
		-	-	(5)[a]
ΔrecU	8.35	780 (11)	1589 (81)	809
		1128 (23)	1867 (41)	739
		954 (35)	1589 (81)	635
		1128 (23)	1729 (23)	601
		1407 (36)	1867 (41)	460
	10.12	1128 (23)	1771 (26)	643
		1128 (23)	1589 (54)	461
		1250 (26)	1525 (17)	275
		1334 (11)	1589 (54)	255
		1356 (14)	1589 (54)	233
	14.52	1335 (20)	1690 (14)	355
		1335 (20)	1648 (11)	313
		1335 (20)	1555 (17)	220
		1335 (20)	1522 (14)	187
		1410 (14)	1555 (17)	145
		-	-	(3)[a]
	17.0	1441	1448	~8
		1441	1445	~4
		1441	1445	~4
		1441	1445	~4
		-	-	(3)[a]

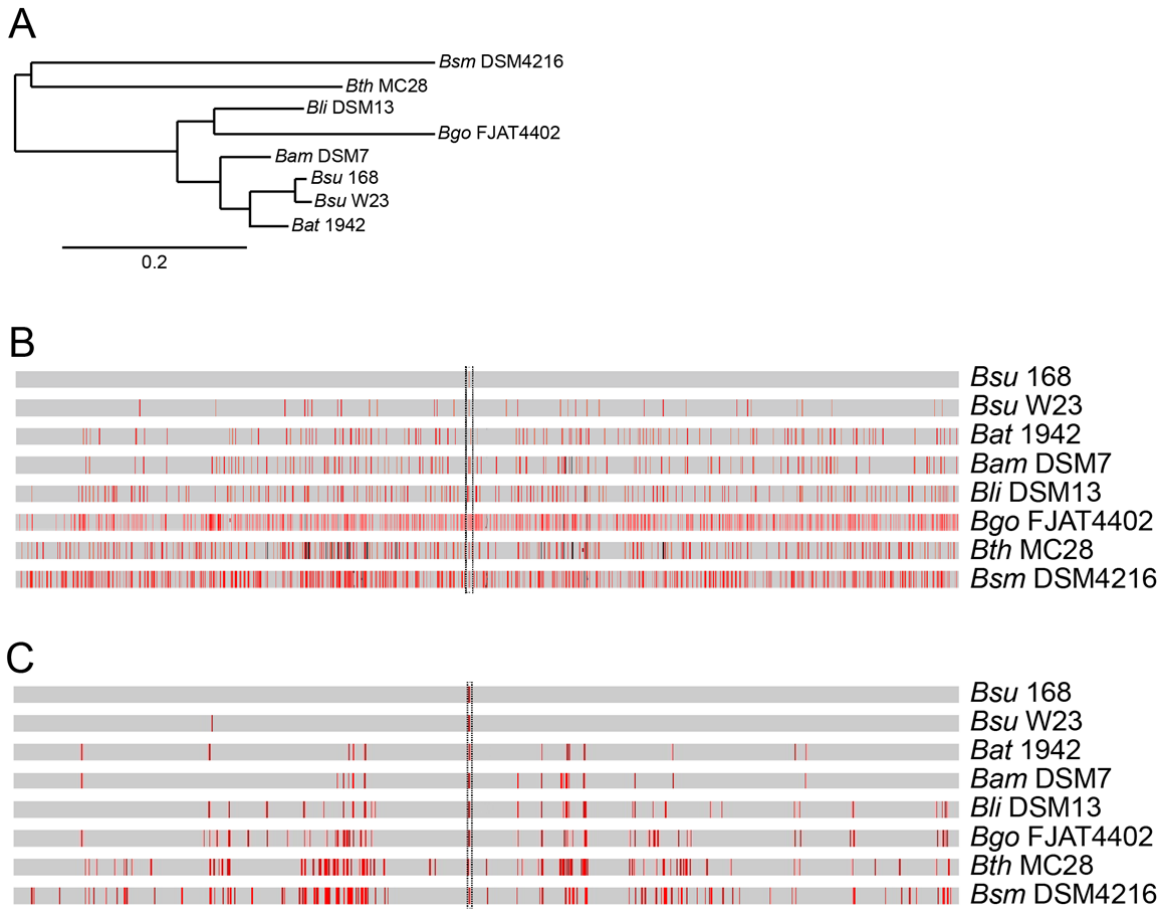
Five Rif^R clones for each condition are documented. [a]The number of spontaneous Rif^R clones analysed (non-genuine transformants) are denoted between parentheses.

Table S2. Mean integration length during interspecies chromosomal transformation in *ΔrecD2*, *ΔrecX*, *ΔradA ΔrecJ* and *ΔdprA* mutants

Genetic background	Divergence (in %)	Left end (MEPS in bp)	Right end (MEPS in bp)	Integration (in bp) [a]
ΔrecD2	8.35	633 (11)	1969 (20)	1336
		1128 (23)	1589 (81)	461
		1394 (11)	2980 (13)	1585
		1370 (36)	1589 (81)	219
		-	-	(13)[a]
	10.12	805 (29)	1589 (54)	784
		1151 (20)	1589 (54)	438
		1371 (14)	1589 (54)	218
		1386 (53)	1589 (54)	203
		-	-	(21)[a]
	14.52	1395 (11)	1555 (17)	160
		-	-	(18)[a]
	17.0	-	-	(25)[a]
ΔrecX	8.35	1152 (23)	1729 (23)	574
		1116 (12)	1589 (81)	473
		1407 (36)	1867 (41)	460
		1344 (20)	1589 (81)	245
		-	-	(5)[a]
	10.12	1251 (26)	1589 (54)	338
		1251 (26)	1525 (17)	274
		1335 (20)	1615 (11)	280
		1371 (14)	1525 (17)	154
		-	-	(12)[a]
	14.52	-	-	(25)[a]
ΔradA	8.35	1 (158)	1909 (23)	1909
		780 (11)	1589 (81)	809
		792 (50)	1589 (81)	797
		954 (35)	1729 (23)	775
		954 (35)	1589 (81)	635
	10.12	1371 (14)	1589 (54)	218
		-	-	(22)[a]
	14.52	-	-	(30)[a]
ΔrecJ	8.35	407 (77)	1774 (11)	1367
		1152 (23)	1589 (81)	437
		1370 (23)	1589 (81)	219
		1370 (23)	1729 (23)	359
		1370 (23)	1639 (17)	269
		-	-	(5)[a]
	10.12	1438 (53)	1589 (54)	151
		-	-	(20)[a]
	14.52	-	-	(25)[a]
ΔdprA	8.35	1128 (23)	1867 (41)	739
		954 (35)	1589 (81)	635
		1251 (14)	1774 (11)	523
		1407 (36)	1867 (41)	460
		-	-	(6)[a]
	10.12	-	-	(25)[a]
	14.52	-	-	(25)[a]
	17.0	-	-	(20)[a]

When available 5 Rif^R clones for each condition are documented. [a]The number of spontaneous Rif^R clones analysed (non-genuine transformants) are denoted between parentheses.

Figure S1



Supplementary Figure 1. Distribution of sequence divergence among different *Bacillus* species. (A) Phylogenetic tree of the selected *Bacillus* species or subspecies based on the *rpoB* nucleotide sequence. Branching confidence values are based on 1,000 bootstrap replicates. (B) Nucleotide sequence differences between the different *Bacilli* genes. A single C-to-T transition mutation at codon 482 (framed by dotted lines), which is centrally located (at position 1443) in the *rpoB*482 gene confers Rif^R. The *rpoB*482 DNA was derived from *B. subtilis* 168 (*Bsu* 168), *B. subtilis* W23 (*Bsu* W23), *B. atrophaeus* 1942 (*Bat* 1942), *B. amyloliquefaciens* DSM7 (*Bam* DSM7), *B. licheniformis* DSM13 (*Bli* DSM13), *B. gobiensis* FJAT-4402 (*Bgo* FJAT4402), *B. thuringiensis* MC28 (*Bth* MC28) and *B. smithii* DSM4512 (*Bsm* DSM4216). Mismatches between donor and recipient are indicated by vertical red bars, and insertions/deletions by vertical black bars. Bar thickness represents the number of mismatches in the neighbourhood. (C) Sequence alignment of the RpoB protein from *Bsu* 168, *Bsu* W23, *Bat* 1942, *Bam* DSM7, *Bli* DSM13, *Bgo* FJAT4402, *Bth* MC28 and *Bsm* DSM4216. The proteins have a high degree of sequence identity (grey). Conserved replacements are indicated by vertical red bars and non-conserved residues by black bars. The alignments were performed using BLAST.