Supplemental material

Why does pfam04002 correspond only to the C-terminus of RadC? Whereas RadC is a ~220-residue widespread bacterial protein (COG2003), the conserved domain called pfam04002 corresponds solely to the C-terminal moiety of the protein. This is due to the fact that the limits of *Escherichia coli radC* were originally defined through complementation of the *radC102* mutation. A *radC102* complementing region was localized on a 1.2-kb BglII/BglII DNA fragment which was sequenced (7). The sequenced complementing fragment contained a potential translation start codon (ATG) followed by an open reading frame of 297 base pairs corresponding to a predicted polypeptide of Mr 11,500. The two protein entries (ACCESSIONs CAA44967 and CAA44966) in the database defined on this basis represent truncated versions (99 residue long) of the full-size RadC protein (222 residues) of *E. coli* K12 (ACCESSION NP_418095).

There is a need to redefine the limits of pfam04002. Since it has been unambiguously demonstrated by Lombardo and Rosenberg (9) that the *radC102* mutation is in fact an allele of *recG*, it would be appropriate to simply remove ACCESSIONs CAA44967 and CAA44966 from the database.

The limits of pfam04002, now DUF2466, should also be redefined so as to match the full-size RadC protein of *E. coli* because this protein aligns throughout its entire length with the orthologous protein of *Streptococcus pneumoniae* and its many bacterial orthologues (data not shown).

Analysis of the fate of transforming DNA. 2 ml *S. pneumoniae* cultures (10^8 cfu/ml) were pre-incubated at 37°C for 2 min and competence was induced with synthetic CSP1 (8) (100 ng/ml) for 15 min at 37°C. Competent cells were then incubated for 3 min at 30°C with 9 µl of a 7771-bp *S. pneumoniae* fragment uniformly labeled with ^{32}P (500 ng; 1.56 10^6 cpm). This fragment was generated by PCR-amplification in the presence of [$\alpha^{32}P$]-dATP, using as template R800 chromosomal DNA and the BM37–AM15 primer pair (Table S1) as previously described (3) but with the Phusion polymerase (Ozyme). Uptake was terminated with DNase I (50 µg/ml; 100 Kunitz Units/ml) and incubation was continued for 1 min. To cool-down the culture, 8 ml of cold CAT medium were then added before centrifugation for 10 min at 10,000 g to harvest cells. The pellet was resuspended in 200 µl SEDS and cells were lysed as described (3). Two phenol extractions followed by ethanol precipitation were used to recover total DNA which was resuspended in 50 µl Tris buffer (10 mM, pH 8.5).

DNA was submitted to overnight electrophoresis (30-35 V/cm) on 30-cm-long 1% agarose gel in Tris-acetate/EDTA buffer. Gels were dried for 2 h at 53°C before exposure to a phosphorimager screen (Fuji Photo Film). Electrophoregrams were analyzed using the MultiGauge software (Fujifilm).

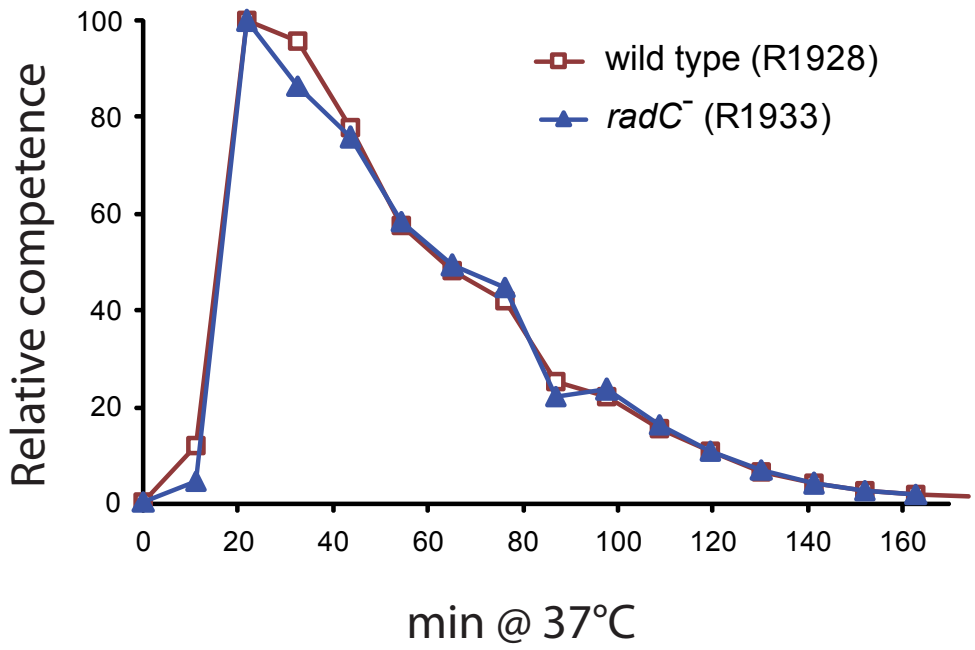

FIG. S1. Kinetics of competence induction and decay in wildtype and *radC* mutant cells. The possible effect of *radC* inactivation on the kinetics of competence induction and decay was assessed by comparing expression of a competence-induced gene, *ssbB*, in wildtype and *radC* mutant cells. Expression of *ssbB* was monitored using a transcriptional fusion with the *Photinus pyralis luc* gene encoding firefly luciferase, the activity of which can be measured directly in living *S. pneumoniae* cells as previously described (Prudhomme and Claverys, 2007). Cultures grown in C+Y medium (initial pH 7.0) to OD_{492}=0.12 were centrifuged, resuspended in $1/10^{th}$ initial volume, and kept frozen (-80°C). To monitor the response to CSP, cells were thawed, diluted 10-fold in C+Y (pH 7.9), and 280 µl were deposited in a 96-well plate. 20 µl of luciferin (2.7 mg/ml) and 0.3 µl CSP (100 µg/ml) were added. Luminescence, which reflects luciferase activity and reports on competence, and OD_{492} were measured during incubation at 37°C. Maximum luminescence (expressed in relative luminescence units, RLU/OD) was 2567 and 2855 RLU/OD, respectively for strains R1928 (wild type) and R1933 (*radC* mutant).

For both strains, *ssbB* expression reached its highest point ~20 min after CSP addition. Competence decay appeared very similar in both wildtype and *radC* mutant cells as judged from the decrease in *ssbB* expression.

These data suggest that RadC plays no role in the response to CSP and in the decay of CSP-induced competence.

FIG. S2. Spontaneous competence induction in wildtype and *radC* mutant cultures.

It is well known that spontaneous competence induction is strongly dependent on the initial pH of the culture medium. For instance, initial pH values between 6.8 and 8.0 affected the timing of the occurrence and the level of competence (5). Therefore, to assess the possible effect of *radC* inactivation on the kinetics of spontaneous competence induction, we compared competence induction under a wide range of initial pH values.

Competence was monitored using the *ssbB*::*luc* transcriptional fusion (see legend to Fig. S1). Luciferase activity (symbols and solid lines) and OD_{492} (dotted lines) were measured in cultures of strain R1848 (wild type; dark blue diamonds) and strain R1932 (*radC* mutant; pink squares) grown at 37°C. For each strain, two parallel cultures were inoculated ($1/100^{th}$ dilution) in C+Y medium at different initial pH (as indicated) from precultures grown in acidic C+Y medium to OD_{492}=0.4. Standard deviation between culture pairs is indicated by error bars.

No significant difference is observed between the two strains suggesting that RadC plays no role in the control of spontaneous competence development in *S. pneumoniae*.

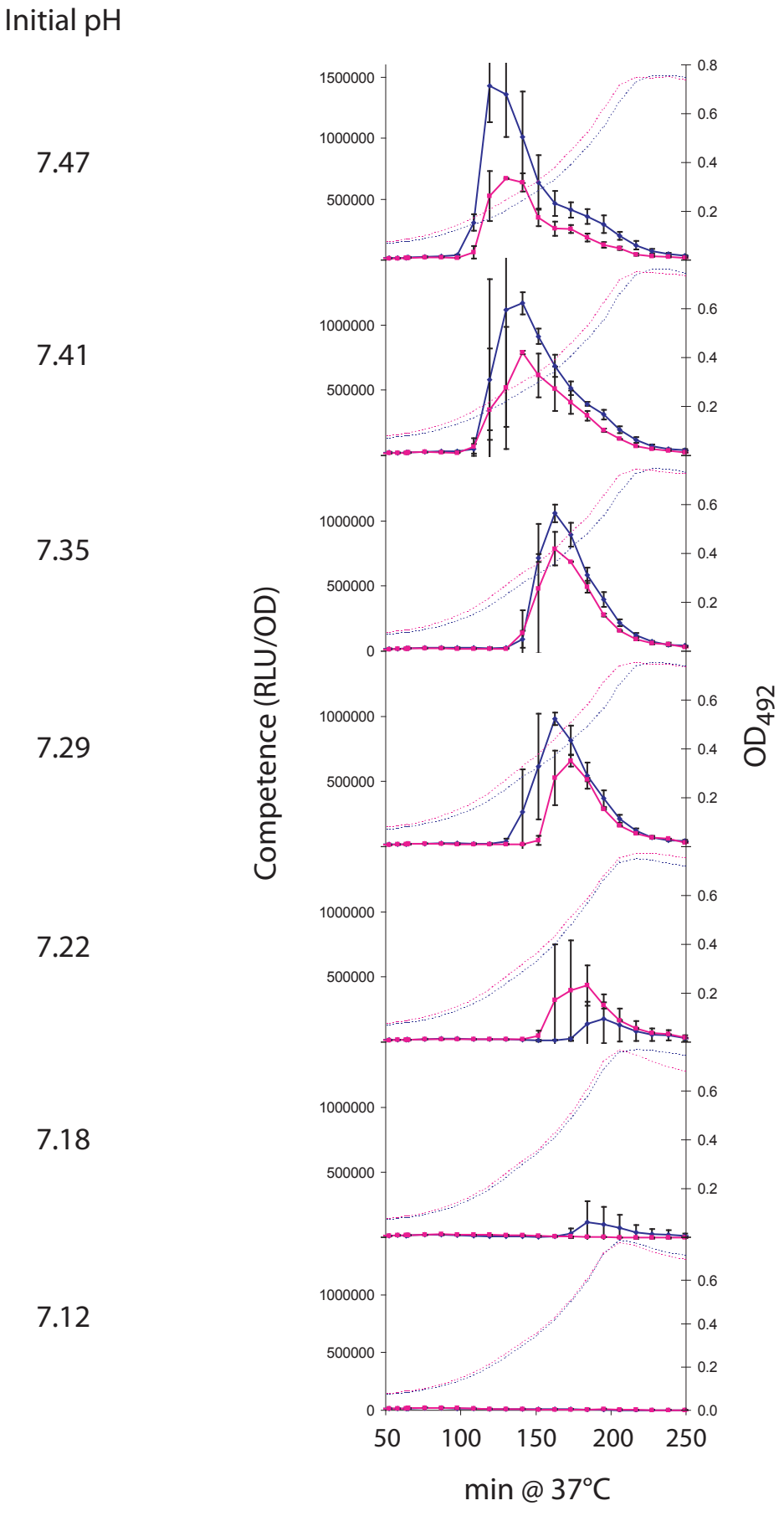

Fig. S2. Attaiech *et al*.

Table S1. Strains, plasmids, and primers used in this study

Strains	**Genotype/description**	**Source/reference**
Streptococcus pneumoniae		
R304	R800 derivative, *nov1*, *rif23*, *str41*; Nov^R, Rif^R, Sm^R	(11)
R800	R6 derivative	(10)
R990	R800 but *rpsl1*; *cbpD*::*kan-rpsL*$^+$; Sm^S, Kan^R	(15)
R1409	R800 but *comA*::*kan*^{42A} (12)*, *ssbB*::pR424 (*luc*), *ssbB*$^+$, *recA*::*ermAM*; Cm^R, Ery^R, Kan^R	This study
R1501	R800 but Δ*comC*	(6)
R1502	R1501 but *ssbB*:: pR424 (*luc*), *ssbB*$^+$; Cm^R	(6)
R1750	R800 but *comA*::*kan*^{C} (2), *ssbB*::pR424 (*luc*), *ssbB*$^+$, *hexAΔ3*::*ermAM*, *comEC*^{H562A}, *dprA*::*spc*^{21C} ; Cm^R, Ery^R, Kan^R, Spc^R	This study
R1848	R800 but *ssbB*::pR424 (*luc*), *ssbB*$^+$, *hexAΔ3*::*ermAM*, *comEC*^{H562A}; Cm^R, Ery^R	This study
R1928	R1502 but *hexAΔ3*::*ermAM*, *comEC*^{H562A}; Ery^R, Cm^R	This study
R1932	R1848 but *radC*::*spc*^{4C} ; Ery^R, Cm^R, Spc^R	This study
R1933	R1928 but *radC*::*spc*^{4C} ; Ery^R, Cm^R, Spc^R	This study
R1939	R1750 but *radC*::*spc*^{4C} ; Ery^R, Cm^R, Spc^R, Kan^R	This study
R1966	R1501 but *radC*::*spc*^{4C}; Spc^R	This study
R1969	R1502 but *radC*::*spc*^{4C}; Cm^R, Spc^R	This study
R2327	R990 but *radC*::*spc*^{4C}; Sm^S, Kan^R, Spc^R	This study
Plasmids		
pR290	pR201 (1) derivative carrying a 1596 bp *Bgl*II-*Hin*dIII genomic fragment (position 1104584-1106179; R6 genome accession number AE007317); Amp^R, Ery^R	This study
pR412	pEMcat derivative carrying a Spc^R (*aad9* gene) *mariner* minitransposon; Amp^R, Spc^R	(13)
pR424	ColE1 derivative carrying an *S. pneumoniae* 5′-*ssbB* targeting fragment adjacent to *luc*; Amp^R, Cm^R	(4)
pLS1	pMV158 derivative, replicative plasmid (rolling-circle type); Tet^R	(14)
pLS70	pLS1 derivative carrying a 3486 bp *Pst*I genomic fragment (position 1897751-1901236, R6 genome accession number AE007317); Tet^R	(14)
Primers	**Sequence; gene; position**[§]	
AM15	TCGTATCATCAACCAAAAGATTGCT; downstream from the *ami* operon (AE005672)	This study

BM37	GGAATTCGGATCCTAAACTCCAAAGTTTCTGCGTC ; upstream from the *ami* operon (AE005672)	(3)
MP127	CCGGGGACTTATCAGCCAACC; mariner transposon	(13)
MP128	TACTAGCGACGCCATCTATGTG; mariner transposon	(13)
radC1	TCGTACCAATTATAACCGTCCGA; *radC*; -464	This study
radC2	CGATTGGTGATCAAGAAATGGCC; *radC*; +1237	This study

[§]Position is given with respect to the ATG of the corresponding gene; - and + indicate upstream and downstream, respectively
[C] and [A] indicate respectively the co-transcribed and the reverse orientation of an inserted mini-transposon antibiotic resistance gene with respect to the targeted gene
*minitransposon inserted at position 94 with respect to the ATG of *comA*

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