Characterization of the *recA* gene from *Pseudomonas fluorescens* OE 28.3 and construction of a *recA* mutant

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The *recA* gene of *Pseudomonas fluorescens* OE 28.3 was isolated by complementation of the Fec^- phenotype of recombinant lambda EMBL3 phages in a RecA^- *Escherichia coli* strain. The subcloned *recA* restored resistance to UV and methyl methanesulphonate (MMS) exposure in *recA* mutants of *E. coli*. DNA sequence analysis showed that the coding region of the *P. fluorescens* gene, specifying a protein of 352 amino acid residues, was preceded by an SOS box highly similar to those of *Pseudomonas aeruginosa* and *Azotobacter vinelandii*. The deduced amino acid sequence displayed highest homology to the RecA proteins from *P. aeruginosa* (87.8% identity) and *A. vinelandii* (84.3% identity). In both the regulatory region and the structural gene, a relatively high degree of sequence divergence from the *Pseudomonas cepacia* gene was observed. A mutant of *P. fluorescens* was constructed by inserting a kanamycin resistance cassette into its *recA* gene. This mutant exhibited an increased sensitivity to UV irradiation and MMS, and was strongly impaired in homologous recombinational activity.

Introduction

Certain soil bacteria occurring in the rhizosphere of crop plants exert a beneficial effect on the development of their hosts by either a direct (hormone production) or an indirect (biocontrol of soil phytopathogens) mechanism (Lugtenberg *et al.*, 1991). In view of the agronomic potential of inocula based on such plant-growth-promoting rhizobacteria (PGPR) for environmentally friendly cropping practices, interest in these bacteria has increased steadily during the last decade, providing new insights into the mechanisms of growth promotion and root colonization. In temperate soils, the fluorescent *Pseudomonas* species *P. fluorescens* and *P. putida* represent the major PGPR.

Recently, we identified a root adhesin in the wheat rhizobacterium *P. fluorescens* OE 28.3 (De Mot & Vanderleyden, 1991), and characterized the correspond-

ing *oprF*-like gene, encoding a major outer-membrane protein (De Mot *et al.*, 1992). Studying the role of this adhesin in root colonization by PGPR requires gene manipulation in *P. fluorescens*. For this purpose, the availability of a recombination-deficient mutant is essential.

The *recA* gene product plays a pivotal role in bacterial homologous recombination, as well as in other cellular processes such as DNA repair (Miller & Kokjohn, 1990; Roca & Cox, 1990). Within the *Pseudomonas* group, *recA* mutants have been characterized for the opportunistic human pathogen *P. aeruginosa* (Früh *et al.*, 1983) and for the foliar pathogen *P. syringae* (Willis *et al.*, 1988; Barbé *et al.*, 1991), both belonging to the same rRNA cluster as *P. fluorescens* (group I). In addition, the *recA* genes of *P. aeruginosa* (Sano & Kageyama, 1987) and of the rRNA group II species *P. cepacia* (Nakazawa *et al.*, 1990) have been sequenced.

In this paper we describe the molecular cloning and sequencing of the *recA* gene from *P. fluorescens* OE 28.3, and the construction and characterization of a RecA^- deficient strain for this wheat isolate.

Methods

Bacterial strains, plasmids, phages, media and growth conditions. The bacterial strains, plasmids and phages used in this work are listed in Table 1. *E. coli* and *P. fluorescens* strains were grown in Luria broth

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Abbreviations: MMS, methyl methanesulphonate; PGPR, plant-growth-promoting rhizobacteria.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number M96558.

Table 1. *Bacterial strains, plasmids and phages*

Relevant characteristics or genotype		Source or reference
<i>P. fluorescens</i>		
OE 28.3	Isolate from wheat rhizosphere	De Mot & Vanderleyden (1991)
FAJ2025	<i>recA</i> mutant of strain OE 28.3	This paper
<i>E. coli</i>		
RR1	<i>proA2 lacY1 hsdS20 endA rpsL20 ara-14 galK2 xyl-5 mtl-1 supE44</i>	Sambrook <i>et al.</i> (1989)
HB101	<i>recA13</i> mutant of strain RR1	
DH1	<i>hsdR17 endA1 thi-1 gyrA96 relA1 recA1 supE44</i>	Simon <i>et al.</i> (1983)
DH5 α	<i>hsdR17 endA1 thi-1 gyrA96 relA1 recA1 supE44 ΔlacU169(ϕ80lacZΔM15)</i>	
S17-1	F ⁻ <i>recA hsdR</i> with modified RP4 in chromosome	
Plasmids		
pKN3	pBR322 with <i>P. cepacia recA</i> gene	Nakazawa <i>et al.</i> (1990)
pSUP102	Cm ^r Tc ^r Mob ⁺	Simon <i>et al.</i> (1983)
pUKA800	pUC8 with Km ^r GenBlock from pUC-4K (Pharmacia)	This laboratory
pFAJ2006	pUC19 with <i>P. fluorescens</i> OE 28.3 <i>recA</i> on 3.8 kb <i>Eco</i> RI fragment	This paper
pFAJ2009	pUC19 with <i>P. fluorescens</i> OE 28.3 <i>recA</i> on 3 kb <i>Hind</i> III fragment	
pFAJ2058	pSUP102 with <i>Hind</i> III fragment from pFAJ2009 inserted in Tc ^r region	
pFAJ2059	pFAJ2058 with Km ^r cassette from pUKA800 inserted in unique <i>Bbr</i> PI site of <i>P. fluorescens recA</i>	
pFAJ2083	pSUP102 with randomly cloned 4 kb <i>Eco</i> RI fragment from strain OE 28.3	
pFAJ2084	pSUP102 with randomly cloned 5 kb <i>Eco</i> RI fragment from strain OE 28.3	
<i>Lambda phages</i>		
λ FAJ2001	λ EMBL3 carrying <i>P. fluorescens</i> OE 28.3 <i>oprF</i> gene	De Mot <i>et al.</i> (1992)
λ FAJ2003	λ EMBL3 carrying <i>P. fluorescens</i> OE 28.3 <i>recA</i> gene	This paper

(LB) medium (37 °C) and trypticase soy broth (TSB) medium (30 °C), respectively. For solid media, 15 g agar l⁻¹ was added. In conjugation experiments, M9 minimal medium was used for selection of *P. fluorescens* transconjugants. Antibiotics were used at the following concentrations: ampicillin 100 µg ml⁻¹, kanamycin 50 µg ml⁻¹, chloramphenicol 25 µg ml⁻¹, tetracycline 10 µg ml⁻¹. α -Complementation of *lacZ* was detected on LB plates supplemented with 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside and isopropyl β -D-thiogalactopyranoside at 20 µg ml⁻¹. Phage-infected *E. coli* cells were plated as described by Frischauf *et al.* (1987). Cell doubling times were estimated from the increase in OD₆₀₀ during aerobic incubation in TSB medium on a rotary shaker (200 r.p.m.).

Determination of sensitivity to UV irradiation and methyl methane-sulphonate (MMS). *E. coli* or *P. fluorescens* cells were grown in LB (37 °C) or TSB (30 °C) to an OD₆₀₀ of about 1. The cells were washed and diluted to approximately 5 $\times$ 10⁷ cells ml⁻¹. Aliquots (100 µl) of several tenfold dilutions were spread in triplicate on LB or TSB plates. When testing MMS sensitivity, the media contained 0.1, 0.5, 1 or 2 mM of this DNA-methylating agent. Different doses of UV radiation (312 nm), ranging from 50 to 200 J m⁻², were applied to the plated cells using a transilluminator. Plates were incubated overnight (or longer when delayed growth was observed in the presence of MMS) at 37 °C (*E. coli*) or 30 °C (*P. fluorescens*). The level of resistance was calculated from the relative number of surviving cells using MMS-free or non-irradiated plates as a control.

DNA methods and sequence analysis. *E. coli* plasmids were extracted using the alkaline lysis method, whereas total DNA from *Pseudomonas* strains was isolated with the cetyltrimethylammonium bromide procedure (Sambrook *et al.*, 1989). Phage DNA was prepared according to Lockett (1990). Double-stranded DNA sequencing of pUC19 subclones was carried out using the AutoRead Sequencing Kit (Pharmacia-LKB) on an automated sequencer (A.L.F., Pharmacia-LKB). Sequence data were processed using the ASSEMBLY program (PC Gene, IntelliGenetics). The PC Gene software was also used for

sequence comparison of deduced proteins by pairwise (ALIGN) or multiple (CLUSTAL) alignments.

Southern hybridization. Genomic or plasmid DNA cut with appropriate restriction endonucleases was transferred by capillary blotting to Hybond-N membranes (Amersham) after agarose electrophoresis. The following probes were isolated by preparative electrophoresis and non-radioactively labelled with the random priming method (DIG chemiluminescent system, Boehringer Mannheim): the 1.2 kb *Eco*RI fragment from pKN3 carrying most of the *P. cepacia recA* gene; the 1.25 kb *Hind*III fragment containing the kanamycin resistance cassette from pUKA800; and the 1.8 kb *Eco*RI–*Bam*HI fragment from pSUP102. The non-radioactive hybridization was carried out as recommended by the manufacturer using a hybridization temperature of 68 °C and final washing in 0.1 $\times$ SSC plus 0.1 % SDS. SSC (1 $\times$) contains 0.15 M-NaCl and 15 mM-sodium citrate at pH 7.0.

Homologous recombination. Following random cloning of genomic *Eco*RI fragments from *P. fluorescens* OE 28.3 in pSUP102, two clones with inserts of 4 kb (pFAJ2083) and 5 kb (pFAJ2084) were selected to assess the homologous recombinational activity in strains OE 28.3 and FAJ2025 by the method of Selbitschka *et al.* (1991). The recombinant plasmids were introduced into these *Pseudomonas* strains by biparental conjugation using *E. coli* S17-1 as a donor. The frequency of appearance of tetracycline-resistant transconjugants per acceptor cell was determined.

Results and Discussion

Cloning of the *recA* gene

A previously constructed genomic library of *P. fluorescens* OE 28.3 in lambda EMBL3 (De Mot *et al.*, 1992) was plated on *E. coli* HB101 cells. Ten plaque-

Fig. 1. Restriction map of the 3.8 kb *EcoRI* fragment of *P. fluorescens* OE 28.3 carrying the *recA* gene. The sequencing strategy is outlined by the arrows and the position of the open reading frame is shown. Restriction sites are indicated as follows: A, *AvaI*; B, *BamHI*; E, *EcoRI*; H, *HindIII*; K, *KpnI*; P, *PstI*; S, *SalI*; V, *EcoRV*.

forming recombinant phages were purified by repeated plating and finally checked for lytic activity on other *RecA*⁻ strains of *E. coli* (DH1, DH5 α). The identical *SalI* restriction patterns of these clones indicated that all of them contained the same insert. The *P. cepacia* *recA* gene was used to probe a Southern blot of DNA from a selected phage (λ FAJ2003) restricted with several endonucleases. For a 3.8 kb *EcoRI* and a 3 kb *HindIII* fragment strong signals were obtained. These fragments were subcloned in pUC19 to generate pFAJ2006 and pFAJ2009, respectively.

A recombinant phage, containing an unrelated DNA fragment (λ FAJ2001; De Mot *et al.*, 1992), was plated on *E. coli* DH5 α cells containing pFAJ2006 or pFAJ2009. Whereas λ FAJ2001 was incapable of lytic growth on strain DH5 α (or other *RecA*⁻ strains such as HB101 and DH1), its plaque-forming ability was restored by the presence of the recombinant plasmids, intermediate to the level obtained with *RecA*⁺ strains. These observations indicated that a functional *recA* gene had been cloned. This was further substantiated by the enhanced UV resistance and reduced MMS sensitivity of *RecA*⁻ *E. coli* hosts carrying pFAJ2006 or pFAJ2009, approaching the levels attained by the *RecA*⁺ strain RR1 (results not shown).

Thus, the *recA* gene from *P. fluorescens* was isolated from a phage library by interspecific complementation of the *Fec*⁻ phenotype of the recombinant phage in *E. coli*. Recently, this approach proved equally successful for the *recA* gene from *Rhizobium phaseoli* (Michiels *et al.*, 1991; Martínez-Salazar *et al.*, 1991). Whereas most *recA* genes have been identified by the ability to confer resistance to DNA damaging agents when cloned in *RecA*⁻ *E. coli* strains, the direct screening of a phage library on a *RecA*⁻ host provides a straightforward alternative method for their identification.

Nucleotide sequence of the *recA* gene

Physical mapping of the inserts from pFAJ2006 and pFAJ2009 showed that the *EcoRI* fragment encompassed the *HindIII* fragment (Fig. 1). The approximate position of the *recA* gene within the latter

fragment was established by Southern hybridization. For the region of interest, several overlapping pUC19 subclones were isolated and sequenced as shown in Fig. 1.

The region sequenced (1444 bp, G+C content 57%) revealed one large open reading frame spaced five nucleotides from a typical Shine–Dalgarno sequence, GAGGA (Fig. 2). Further upstream of the coding region (positions 100–119), the sequence TACTGTCTACTTATACAGGT was identified as a putative SOS box, corresponding to the *E. coli* consensus sequence CTG-N₁₀-CAG that enables binding of the LexA repressor protein (Walker, 1987). This *P. fluorescens* SOS box most closely resembles those reported for *P. aeruginosa* and *Azotobacter vinelandii* (Fig. 3). Only one single base out of 20 is different between these three species. Higher sequence divergence is seen for another *Pseudomonas* species, *P. cepacia*, five and six bases being different from *P. aeruginosa* and *P. fluorescens*, respectively. Surprisingly, the *P. cepacia* SOS box shows closer resemblance to the one from *Bordetella pertussis* (differences at four positions only).

Partially overlapping with the putative repressor-binding region of *P. fluorescens* *recA*, a putative σ^{70} -type promoter sequence is found between positions 89–116 (Fig. 3). This TTGTGG-N₁₆-TATACA sequence is similar to known σ^{70} -dependent *Pseudomonas* promoters (Deretic *et al.*, 1989). In the regulatory region of the *recA* gene from *Pseudomonas aeruginosa*, the sequence CTGTGG-N₁₆-TATACA is present, differing only at the first position (Sano & Kageyama, 1987). Despite the high homology between their SOS boxes (covering the -10 promoter region in both fluorescent *Pseudomonas* species), no obvious *Pseudomonas*-like -35 region appears to occur in *A. vinelandii* (Venkatesh & Das, 1992). However, a related -35/-10 sequence (TTGCAC-N₁₅-TATACA) partially overlaps with the SOS box from *P. cepacia* (Nakazawa *et al.*, 1990) and is reminiscent of the motif found in *B. pertussis*: TGGCAC-N₁₅-AGTACA (Favre & Viret, 1990). A promoter region overlapping with a potential LexA binding site also exists in *Vibrio anguillarum* (Tolmasky *et al.*, 1992).

These observations indicate that a LexA-like mediated SOS response is probably operative in several bacteria outside the *Enterobacteriaceae* (Zhao & McEntee, 1990), including fluorescent *Pseudomonas* species. Actually, this was recently shown by introducing a broad-host-range plasmid, carrying an *E. coli* *recA-lacZ* operon fusion, into various Gram-negative bacteria (Fernandez de Henestrosa *et al.*, 1991). Repression and induction of *recA* transcription in, respectively, the absence and presence of the DNA damaging agent mitomycin C was demonstrated in, among other bacteria, *P. syringae*, *P.*

GTCGACAAACGGTAAAGGCCGCTGGAGGGCTTGTTCGACGAGCTGCAGCAGAAATCGAAAATCAGGGGT 72
 AGGCGATCCCGGATCTTTGTGGAACAATFACTGTCTACTTATACAGGTTTGGCCGTCAGCTTATTGATTACG 144
 TGAGGACTTTAATGGACGACAACAAGAAGAAAGCCTTGGCTGCGGCCCTGGGTGAGATCGAACGTCAATTCC 216
 M D D N K K K A L A A A L G Q I E R Q F
 GCAAGGGTGCCGTAATGCGTATGGGCGATCACGACCGTCAAGCGATCCCGGCTATTTCCACTGGCTCTCTGG 288
 G K G A V M R M G D H D R Q A I P A I S T G S L
 GTCTGGACATCGCACTCGGCATTGGCGGCCTGCCAAAAGGCCGTATCGTTGAAATCTACGGTCCTGAATCTT 360
 G L D I A L G I G G L P K G R I V E I Y G P E S
 CCGGTAAACCACCTTGACCCTGTCGGTGATTGCCCAAGCGCAAAAAATGGGCGCCACCTGTGCGTTTCGTGCG 432
 S G K T T L T L S V I A Q A Q K M G A T C A F V
 ACGCCGAGCAGCCCTTGACCCGGAATACGCCGGTAAGCTGGGCGTCAACGTTGACGACCTGCTGGTTTTCCC 504
 D A E H A L D P E Y A G K L G V N V D D L L V S
 AGCCGGACACCGGTGAGCAAGCCCTGGAAATCACCGACATGCTGGTGCCTCCAACGCCATCGACGTGATCG 576
 Q P D T G E Q A L E I T D M L V R S N A I D V I
 TGGTCGACTCCGTGGCTGCCCTGGTACCGAAAGCTGAAATCGAAGGCGAAATGGGCGACATGCACGTGGGCC 648
 V V D S V A A L V P K A E I E G E M G D M H V G
 TGCAAGCCCCGCTGATGTCCCAGGCGCTGCGTAAATACCGGTAACATCAAGAACGCCAACTGCCTGGTGA 720
 L Q A R L M S Q A L R K I T G N I K N A N C L V
 TCTTCATCAACCAGATCCGTATGAAGATCGGCGTAATGTTTCGGCAGCCCCGGAACCACTACCGGTGGTAACG 792
 I F I N Q I R M K I G V M F G S P E T T T G G N
 CGCTGAAGTTCTACGCTTCGGTCCGTCTGGACATCCGCCGTACCGGCGCGGTGAAGGAAGGTGACGAAGTTG 864
 A L K F Y A S V R L D I R R T G A V K E G D E V
 TTGGTAGCGAAACTCGCGTTAAAGTCGTGAAGAACAAGGTGCGTCCGCCCTTTCCGTCAGGCAGAGTTCCAGA 936
 V G S E T R V K V V K N K V A P P F R Q A E F Q
 TTCTCTACGGCAAGGGTATCTACCTGAACGGCGAGATGATTGACCTGGGCGTACTGCACGGTTTCGTCGAGA 1008
 I L Y G K G I Y L N G E M I D L G V L H G F V E
 AGTCCGGTGCCTGGTATGCCTACAACGGCAGCAAGATCGGTGAGGCAAGGCCAACTCGGCCAAGTTCTCTGG 1080
 K S G A W Y A Y N G S K I G Q G K A N S A K F L
 CAGACAACCCGGATATCGTCGCCACGCTTGAGAAGCAGATTCGCGACAAGCTGCTGACCCCAGCGCCAGACG 1152
 A D N P D I V A T L E K Q I R D K L L T P A P D
 TGAAAGCTGCCGCCAACCGCGAGCCGGTTGAAGAAGTGAAGAAGCTGACACTGATATCTGAAGCAAACGAT 1224
 V K A A A N R E P V E E V E E A D T D I *
 GACTGTGGTACTGGATACACTCGTCGCGTTCGGCGCACCGCGATGGACCTGCTCGCTCGCCGCGAGCATGG 1296
 TCGAGTCGAGCTGACGCGTAACTGCGTCAGCGCGGTGCAGAGCCCGAGATGATCGAAACAGCCCTCGACCG 1386
 TTTGACGGAAGAGGGGCTGCTTTCCGAAGCCGCTATCTTGAAAGCTTTGTTTCCTACCGCGCGCGCTCCGG 1440
 CTAT 1444

Fig. 2. DNA sequence and deduced amino acid sequence of the *P. fluorescens recA* gene. The putative ribosome binding site and downstream potential stem-loop structures are underlined. In the 5' regulatory region, the SOS sequence is boxed and the -35/-10 regions of a putative σ^{70} -type of promoter are printed in italic type. The arrow points out the unique *Bbr*PI site (CACGTG) used for insertional inactivation of the gene.

<i>Pf</i>	T A C T G T C T A C T T A T A C A G G T
<i>Pa</i>	T A C T G T C T A C T T A T A C A G T T
<i>Az</i>	T A C T G T C T A C T T A T A C A G T G
<i>Pc</i>	C A C T G T T T T T T A T A C A G T G
<i>Bp</i>	C A C T G G A T T T T T A T C A G T A
<i>Ec</i>	T A C T G T A T G A G C A T A C A G T A
<i>Er</i>	T G C T G T A T G C G C A T A C A G T A
<i>Pm</i>	T A C T G T A T G A T T A T A C A G T A
<i>Sm</i>	T A C T G T A T G A C C A T A C A G T A
<i>Va</i>	C A C T G T A T G A A T C A A C A G T A
<i>Rm</i>	C G C T G C C T G T G G A T A C A G T T

Fig. 3. Alignment of the LexA operator sequence, preceding the *Escherichia coli recA* gene (*Ec*), with the putative SOS regulatory elements from *Azotobacter vinelandii* (*Az*), *Bordetella pertussis* (*Bp*), *Erwinia carotovora* (*Er*), *Proteus mirabilis* (*Pm*), *Pseudomonas aeruginosa* (*Pa*), *P. cepacia* (*Pc*), *P. fluorescens* (*Pf*), *Rhizobium meliloti* (*Rm*), *Serratia marcescens* (*Sm*) and *Vibrio anguillarum* (*Va*). The corresponding sequences of *Shigella flexneri* (identical to *E. coli*) and of *Proteus vulgaris* (identical to *P. mirabilis*) are not shown. Boxed regions indicate perfect base conservation throughout currently recognized SOS sequences (Favre & Viret, 1990; Selbitschka *et al.*, 1991; Tolmasky *et al.*, 1992; Venkatesh & Das, 1992).

Fig. 4. Dendrogram showing relative distances between RecA proteins, based on multiple sequence alignment (CLUSTAL program) of the proteins from *Acholeplasma laidlawii* (Al), *Anabaena variabilis* (Av), *Aquaspirillum magnetotacticum* (Am), *Azotobacter vinelandii* (Az), *Bacillus subtilis* (Bs), *Bacteroides fragilis* (Bf), *Bordetella pertussis* (Bp), *Erwinia carotovora* (Er), *Escherichia coli* (Ec), *Methylobacillus flagellatum* (Mf), *Neisseria gonorrhoeae* (Ng), *Proteus mirabilis* (Pm), *Pr. vulgaris* (Pv), *Pseudomonas aeruginosa* (Pa), *P. cepacia* (Pc), *P. fluorescens* (Pf), *Rhizobium leguminosarum* (Rl), *R. phaseoli* (Rp), *R. meliloti* (Rm), *Serratia marcescens* (Sm), *Synechococcus* sp. (Sy), *Thiobacillus ferrooxidans* (Tf) and *Vibrio anguillarum* (Va). The *Shigella flexneri* sequence (not shown) is identical to RecA of *E. coli*. The respective percentages of residues identical with the *P. fluorescens* protein as deduced from pairwise alignments, are indicated on the right. References to sequences not included in the review by Roca & Cox (1990) are: Favre & Viret (1990), Fyfe & Davies (1990), Gomelsky *et al.* (1990), Goodman & Woods (1990), Nakazawa *et al.* (1990), Michiels *et al.* (1991), Selbitschka *et al.* (1991), Dybvig & Woodard (1992), Tolmashy *et al.* (1992) and Venkatesh & Das (1992).

aeruginosa and *P. putida*. From the latter two species, *lexA*-like genes have now been cloned (Calero *et al.*, 1991). In *Bacillus subtilis*, the putative repressor of the SOS-like SOB regulon recognizes an operator sequence different from the LexA box of *E. coli* (Cheo *et al.*, 1991).

Deduced amino acid sequence of RecA

The open reading frame in Fig. 2 encodes a protein of 352 amino acids with a calculated molecular mass of 37634 Da. Since the comparison of RecA sequences by Roca & Cox (1990), several new *recA* genes have been sequenced, including those from some species belonging to quite distant genera such as *Bacteroides* and *Acholeplasma*. For a detailed comparison of *P. fluorescens* RecA with the currently known corresponding proteins from other Eubacteria, a multiple sequence alignment was carried out for 24 sequences in order to construct a RecA-based dendrogram (Fig. 4). The actual sequence alignment is shown in Fig. 5. In these alignments, the amino acid sequence deduced from the *Mycobacterium tuberculosis recA* locus is not included since it contains a large central non-homologous region of 440 residues (Davis *et al.*, 1991).

Apparently, the extent of sequence homology among RecA proteins reflects well the phylogenetic relationship of the respective bacteria. Indeed, nicely separated clusters were generated for enterobacteria, rhizobia and cyanobacteria. The representatives of Gram-positive

bacteria and the related mycoplasmas, as well as of the bacteroides are also well separated (Fig. 4).

The *P. fluorescens* RecA is most similar to the *P. aeruginosa* protein: 87.8% identical residues as compared to 69.5% with *E. coli* RecA (Fig. 4). Surprisingly, RecA from *Azotobacter vinelandii* shares 84.3% of its residues with the *P. fluorescens* protein. As can be deduced from their clustering in Fig. 4, homology between the *Azotobacter* species and *P. aeruginosa* is even higher (89.2% identical amino acids). As mentioned above, this high level of sequence conservation is extended to the upstream regulatory region of these three genes, namely their putative SOS boxes. A substantially lower degree of similarity exists between the *P. fluorescens* and *P. cepacia* RecA proteins (69.8% identity). Conversely, *P. cepacia* RecA shows much better alignment with the *Bordetella pertussis* protein (Fig. 4). The RecA proteins from these species possess as much as 81.2% identical amino acids. As discussed earlier, the rather unexpected clustering of these *Bordetella* and *Pseudomonas* species is again reflected in a high degree of similarity of the respective regulatory regions. These observations add to the uncertainty about the current taxonomic position of *P. cepacia* within the large *Pseudomonas* group.

As more *recA* genes are sequenced, the extremely high degree of sequence conservation among their gene products (23% overall identity) becomes more striking. For instance, *P. fluorescens* RecA has over 56% residues

Az	EAQKQGATCAFVDAEHALDPDYAAKLGVNVDLLVSPQDTGQALEITVMLVRSNAVDVIIVDSVAALVPKAEIEGEMGDQHVGLQARLMSQ	172
Pa	EAQKQGATCAFVDAEHALDPDYAGKLGVNVDLLVSPQDTGQALEITDMLVRSNAVDVIIVDSVAALVPKAEIEGEMGDAHVGLQARLMSQ	172
Pf	QAKMGATCAFVDAEHALDPEYAGKLGVNVDLLVSPQDTGQALEITDMLVRSNAVDVIIVDSVAALVPKAEIEGEMGDHVGGLQARLMSQ	172
Sm	AAQREGKTCAFIDAHAALDPIYAKKLGVDIDLNLLCSQPDGTGEQALEICDALTRSGAVDVIIVDSVAALTPKAEIEGETGDSHMGGLAARMMSQ	173
Ec	AAQREGKTCAFIDAHAALDPIYAKKLGVDIDLNLLCSQPDGTGEQALEICDALRSAGAVDVIIVDSVAALTPKAEIEGETGDSHMGGLAARMMSQ	174
Pm	SAQREGKICAFIDAHAALDPIYAKKLGVDIDLNLLCSQPDGTGEQALEICDALRSAGAVDVIIVDSVAALTPKAEIEGETGDSHMGGLAARMMSQ	173
Pv	AAQREGKICAFIDAHAALDPIYAKKLGVDIDLNLLCSQPDGTGEQALEICDLSRSGAVDVIIVDSVAALTPKAEIEGETGDSHVGGLAARMMSQ	173
Er	AAQREGKTCAFIDAHAALDPIYAKKLGVDIDLNLLCSQPDGTGEQALEICDALTRSGAVDVIIVDSVAALTPKAEIEGETGDSHMGGLAARMMSQ	173
Va	AAQRVGKTCAFIDAHAALDPIYAKKLGVDIDLNLLCSQPDGTGEQALEICDALRSAGAVDVIIVDSVAALTPKAEIEGEMGDSHMGGLQARLMSQ	172
Mf	QMQLGGTAAFDIAEHALDPVYAAKLGVNVDLLVSPQDTGQALEITADMLVRSAGVDVVDVDSVAALTPKAEIEGEMGDSHMGGLQARLMSQ	173
Bp	EMQLGGTCAFVDAEHALDPVYAAKLGVNLTDLVSPQDTGQALEITDALVRSAGVDLVIDVDSVAALVPKAEIEGEMGDSLPGQLQARLMSQ	180
Pc	LQLKLGTTAAFDIAEHALDPVYAAKLGVNPELLISQPDGTGEQALEITDALVRSAGVDLVIDVDSVAALVPKAEIEGEMGDSLPGQLQARLMSQ	173
Tf	SCQAAGGTAAFDIAEHALDPGYAAKLGVDLENLLISQPDGTGEQALEITADMLVRSAGVDLVIDVDSVAALVPKAEIEGEMGDSHVGGLQARLMSQ	173
Ng	QQKNGGVCFAFDIAEHAAPDVYAAKLGKVKEVLYLSQPDGTGEQALEITDTLVRSAGVDVVDVDSVAALVPKAEIEGDMGDSHVGGLQARLMSQ	173
Am	EAQKKGGTCAFVDAEHALDPSYAAKLGVA-LDELLISEPDAGQALEITDTLVRSAGVDVVDVDSVAALVPRGELEGEMGDNMHGLHARLMSQ	170
Rl	EAQKKGGICAFVDAEHALDPVYAAKLGVDLQNLVLSQPDGTGEQALEITDTLVRSAGVDVVDVDSVAALTPRAEIEGEMGDTVPGQLQARLMSQ	173
Rp	ESQKKGGICAFVDAEHALDPVYAAKLGVDLQNLVLSQPDGTGEQALEITDTLVRSAGVDVVDVDSVAALTPRAEIEGEMGDSLPGQLQARLMSQ	181
Rm	EAQKKGGICGFDIAEHALDPVYAAKLGVDLENLLISQPDGTGEQALEITDTLVRSAGVDLVIDVDSVAALVPKAEIEGEMGDSLPGMQARLMSQ	171
Bs	EVQQR- TSAFDIAEHALDPVYAAKLGVNIEELLSPQDTGQALEIAEALVRSAGVDIIVDSVAALVPKAEIEGDMGDSHVGGLQARLMSQ	170
Al	SAQKGGTVAFIDAHAALDPNYAKALGVDLNLVSPQDTGQALDIAEALVRSAGVDIIVDSVAALVPEAEIAGDSANHVGLQARLMSQ	173
Bf	EAQKAGGIAAFIDAHAAPDRFYAAKLGVDVNLFIQPDNGEQALETAEQLRSSAIDIIVDSVAALTPKAEIEGDMGDNKVGGLQARLMSQ	160
Sy	EVQKAGVAAFDIAEHALDPTYSAAALGVQDNIENLLVSPQDNGESALETADQLVRSAGVDIIVDSVAALVPEAEIEGEMGDVQVGLQARLMSK	176
Av	EVQKEGGIAAFVDAEQALDPTYSASALGVDIQNLVLSQPDGTGSALETIVDQLVPSAADVIVDSVAALVPEAEIEGDMGDAHVGLQARLMSQ	176

Fig. 5. For legend see opposite.

in common with distant species such as *Synechococcus* sp. and *Acholeplasma laidlawii* (Fig. 4). In view of these data, it is unlikely that the sequence reported for *Vibrio cholerae* (Ghosh *et al.*, 1992) represents its RecA protein, sharing only 13% of its amino acids with the protein from the rather closely related *E. coli*. Also, no significant alignment with *V. anguillarum* RecA could be achieved. Therefore, the *V. cholerae* sequence was excluded from the multiple alignment and dendrogram building in this paper.

Specific parts of the RecA proteins are most highly conserved (Fig. 5). For the ATP-binding domain proposed by Higgins *et al.* (1986), the consensus sequence [I/V][I/V]E[I/V][Y/F]GPES[S/Y]GKTT[L/V], corresponding to amino acids 60–74 of *P. fluorescens* RecA can now be deduced. Another contiguous, conserved portion is found between residues 202 and 222, reflecting the consensus [F/Y]G[N/S]PE[T/V]T[T/P]G[G/A]XALKF[Y/F][A/S]SVR[L/M]. It is located in a spacer between two core elements of the proposed three-dimensional structure of *E. coli* RecA (Miller &

Kokjohn, 1990). The present compilation of data favours the continued use of part of this sequence (AL ... VR) as a RecA signature (Bairoch, 1991). High sequence conservation is also evident in the N-terminal domain with the consensus sequence [Q/K]I[D/E][K/R]X[F/Y]-GKG[A/S][V/I]M[K/R] (residues 15-27 of *P. fluorescens* RecA), which is essential for interaction with single-stranded DNA and for filament formation (Miller & Kokjohn, 1990). On the other hand, the C-terminal region of *P. fluorescens* RecA shows little sequence homology with its counterparts from other bacteria (Fig. 5). However, it is enriched in acidic residues (7 in a stretch of 13 amino acids), as are most other RecA proteins (Roca & Cox, 1990).

In the deduced consensus sequences, the corresponding regions of the anomalously long RecA from *Mycobacterium tuberculosis* have been included (Davis *et al.*, 1991). Recently, short internal *recA* fragments from several Gram-positive bacteria and mycoplasmas were isolated by using the polymerase chain reaction and sequenced (Duwat *et al.*, 1992; Dvbyvig *et al.*, 1992).

Pa	ALRKITGMIONANCLVIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRTGAVKESDEV-VGSETRVKVVKNKVAPPFRQAEFQILY	
Pf	ALRKITGMIONANCLVIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRTGAVKESDEV-VGSETRVKVVKNKVAPPFRQAEFQILY	
Sm	AMRKLGNLKNANTLLIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRIGAIKEGDEV-VGSETRVKVVKNKIAAPFFKQAEFQIMY	
Ec	AMRKLGNLKNANTLLIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRIGAIKEGDEV-VGSETRVKVVKNKIAAPFFKQAEFQILY	
Pm	AMRKLGNLKNANTLLIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRIGAIKEGDEV-VGSETRVKVVKNKIAAPFFKQAEFQIMY	
Pv	AMRKLGNLKNANTLLIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRIGAIKEGDEV-VGSETRVKVVKNKIAAPFFKQAEFQIMY	
Er	AMRKLGNLKNANTLLIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRIGAIKEGDEV-VGSETRVKVVKNKIAAPFFKQAEFQILY	
Va	AMRKLGNLKNANTLLIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRIGAIKEGDEV-VGSETRVKVVKNKIAAPFFKQAEFQILY	
Mf	ALRKLTANIKRTMTLVIFINQIRMKIGLFGNPETTTGGNALKFYASVRLDIRRTGAIKKGDEV-TGSETRVKVVKNVAPPFFKLAEPDILY	
Bp	ALRKLTATIKRTMTLVIFINQIRMKIAVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Pc	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Tf	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Ng	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Am	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Rl	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Rp	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Rm	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Bs	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Al	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Bf	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Sy	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	
Av	ALRKLTATIKRTMTLVIFINQIRMKIGVFGNPETTTGGNALKFYSSVRLDIRRIGAIKKGDEV-VGNETRVKVVKNKVAPPFFKQAEFDIMY	

Az	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	349
Pa	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	346
Pf	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	352
Sm	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	354
Ec	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	352
Pm	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	354
Pv	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	355
Er	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	324
Va	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	348
Mf	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	344
Bp	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	352
Pc	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	347
Tf	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	346
Ng	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	348
Am	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	344
Rl	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	351
Rp	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	360
Rm	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	348
Bs	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	347
Al	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	331
Bf	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	318
Sy	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	348
Av	GKGIYRNGEIIDLGVLGLLEKSGAWSYSGSKIGQGGKANAALFLDNPEVAAAVEKSIRDQLLAAPASARPAALADEPADADLDY	358

The *recA* mutant of *P. fluorescens* OE 28.3 produced somewhat smaller colonies on solid medium and had a slightly increased doubling time in liquid TSB medium (102 min as compared to 89 min for the wild-type strain). A similar effect on growth rate was reported for *recA*

Fig. 6. Sensitivity of *P. fluorescens* OE 28.3 (●) and its *recA* mutant FAJ2025 (○) to UV irradiation (a) and MMS (b). The fraction of surviving cells (N/N_0) was determined for each treatment from the number of residual cells (N) and the number of cells initially applied (N_0). Values represent means of three determinations. At 2 mM-MMS, no surviving cells of FAJ2025 were detectable.

mutants generated for *Rhizobium meliloti* and *R. leguminosarum* (Selbitschka *et al.*, 1991).

Strain FAJ2025 was distinctly more sensitive to MMS and substantially less resistant to UV irradiation as compared to the parent strain (Fig. 6), which is typically observed for *recA* mutants (Miller & Kokjohn, 1990).

The relative recombinational activity of the *recA* mutant was estimated by determining the integration frequency of the tetracycline resistance marker from the suicide plasmid pSUP102 carrying a large *Pseudomonas* insert. Frequencies were reduced from 9×10^{-4} in the wild type strain to 5×10^{-9} in the mutant in case of pFAJ2083 (4 kb insert), and from 5×10^{-4} to 4×10^{-9} when using pFAJ2084 (5 kb insert). These data clearly demonstrate that the *recA* mutant FAJ2025 is severely impaired in performing homologous recombination.

The recombination-deficient *P. fluorescens* mutant will be useful for further genetic experiments that require a stable maintenance of introduced, plasmid-borne genes to study their involvement in processes such as root adhesion, antibiotic-mediated antagonism of phytopathogens or direct plant growth promotion.

Note added in proof. The deduced amino acid sequence for the *Lactococcus lactis* subsp. *lactis* RecA [Duwat, P., Ehrlich, S. D. & Gruss, A. (1992). Use of degenerate primers for polymerase chain reaction cloning and sequencing of the *Lactococcus lactis* subsp. *lactis* *recA* gene. *Applied and Environmental Microbiology* **58**, 2674–2678] deviates from the N-terminal consensus

sequence presented in this paper at two positions (L instead of Q or K, and L instead of V or I).

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