Cloning and Expression in *Escherichia coli* of a *recA*-like Gene from the Acidophilic Autotroph *Thiobacillus ferrooxidans*

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A recombinant plasmid, pRSR100, containing the functional analogue of the *Escherichia coli* *recA* gene was isolated from a genomic library of *Thiobacillus ferrooxidans* ATCC 33020. The plasmid complemented defects in DNA repair and homologous recombination in *E. coli* *recA* mutant strains. Antiserum raised against *E. coli* RecA protein reacted with the native but defective *E. coli* HB101 RecA protein; it did not react with protein extracts from the *recA* deletion mutant *E. coli* JK696, but it reacted with two protein bands in extracts of *E. coli* JK696(pRSR100). A single band with an apparent $M_r$ equal to the higher-$M_r$ band in *E. coli* JK696(pRSR100) was detected in *T. ferrooxidans* cell extracts with the *E. coli* RecA antiserum.

INTRODUCTION

*Thiobacillus ferrooxidans* is an acidophilic, diazotrophic, autotrophic bacterium that can obtain energy through the oxidation of ferrous iron to ferric iron or reduced inorganic sulphur compounds to sulphuric acid. This unusual physiology enables the organism to grow in inorganic mining environments, where it is used industrially to leach metals from a variety of ores. There is much interest in the molecular biology of *T. ferrooxidans* and efforts to develop a genetic system for the bacterium are in progress.

Recent reports indicate the presence of RecA-like proteins in a wide variety of heterotrophic bacteria (Kokjohn & Miller, 1985; Goldberg & Mekalanos, 1986; Goodman *et al.*, 1987) and at least three cyanobacteria (Owttrim & Coleman, 1987; Geoghegan & Houghton, 1987; Murphy *et al.*, 1987). Cloned *recA* genes from several species have been shown to complement *recA* mutations in *Escherichia coli*. In *E. coli* the *recA* gene product is required for homologous genetic recombination and the induction of the SOS repair system (Walker, 1984). Purified RecA protein possesses both recombinase and protease activity. Recombinase activities include an ATP-dependent assimilation of single-stranded DNA into homologous duplex DNA (Shibata *et al.*, 1981) as well as the pairing and exchange of duplex DNA (West *et al.*, 1981). Protease activities include the cleavage of phage $\lambda$ repressor and the repressor of the SOS system, the LexA protein. Little (1984) suggested that phage $\lambda$ repressor and the LexA protein may be cleaved by autodigestion which is stimulated by the RecA protein.

We investigated whether *T. ferrooxidans* has a RecA-like protein similar to that found in heterotrophic and cyanobacteria. The cloned *T. ferrooxidans recA*-like gene was further characterized for its ability to functionally complement both recombinase and protease activities in *E. coli recA* mutants.

METHODS

*Bacterial strains and bacteriophages.* These are listed in Table 1.

*Media.* *T. ferrooxidans* was grown and maintained on the iron-based medium of Silverman & Lundgren (1959). *E. coli* strains were grown in Luria medium; when required, ampicillin (100 $\mu$g ml$^{-1}$) was added. The DNA-damaging agents MMS and NQO were used to select for the RecA* phenotype. Freshly prepared Luria agar

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Abbreviations: MMS, methyl methanesulphonate; NQO, 4-nitroquinoline-1-oxide; e.o.p., efficiency of plating.
Tris/HCl (pH 7.4), and pRSR102. Cells were mixed and poured onto Luria agar plates. The number of plaques was determined after 18 h incubation at 37 °C.

Antibody binding and the development of bands using a goat anti-rabbit serum conjugated to horseradish peroxidase was done according to the method of Rybicki et al., 1983. Chromosomal DNA was partially digested with Sau3A and the fragments were sized on a sucrose gradient. Fractions containing DNA fragments of 4–10 kb were isolated and ligated into the pBR322 origin of replication. It was derived from the pCL plasmids described by Zabeau et al., 1984). Chromosomal DNA was isolated as described previously (Barros et al., 1984). Chromosomal DNA containing MMS at a final concentration of 0.01% (w/v) was used for initial screening of the recombinant T. ferrooxidans chromosomal library. M9 minimal medium (Maniatis et al., 1982) to which thiamin (5 μg ml⁻¹) proline (20 μg ml⁻¹), leucine (20 μg ml⁻¹) and streptomycin (30 μg ml⁻¹) were added as required was used in bacterial conjugation experiments.

Preparation of DNA and cloning techniques. Cloning techniques were those of Maniatis et al. (1982). Plasmid pEcoR251, a gift from M. M. Zabeau, Plant Genetic Systems, Ghent, Belgium, is a positive selection vector containing the E. coli EcoRI gene under the control of the λ rightward promoter, the ampicillin-resistance gene and the pBR322 origin of replication. It was derived from the pCL plasmids described by Zabeau & Stanley (1982). The EcoRI gene product expressed at high levels by the λ rightward promoter on pEcoR251 is lethal unless insertionally inactivated or regulated by pcl857, which contains a temperature-sensitive λ repressor gene (Remaut et al., 1983). The EcoRI gene has a single BglII cloning site. T. ferrooxidans chromosomal DNA was isolated as described previously (Barros et al., 1984). Chromosomal DNA was partially digested with Sau3A and the fragments were sized on a sucrose gradient. Fractions containing DNA fragments of 4–10 kb were isolated and ligated into the BgII site of pEcoR251.

UV sensitivity. Cells were grown to an OD₆₀₀ of 0.2 in Luria broth, harvested by centrifugation and resuspended in an equal volume of Ringer’s solution. Samples (5 ml) were placed in a Petri dish and irradiated with UV light using a Cole-Palmer series 9815 germicidal lamp. The UV fluence was determined with a Blak-Ray ultraviolet meter (Ultraviolet Products, Inc.). Survivors were determined by plating on Luria agar. All manipulations were carried out under subdued lighting and incubation was done in the dark to prevent photoreactivation.

Bacterial conjugation. Overnight cultures were diluted 1:10 in Luria broth and incubated for 1 h at 37 °C. The cells were mixed in a donor-to-recipient ratio of 1:10 and allowed to conjugate for 60 min at 37 °C, with gentle shaking. Selection for transconjugants was by acquisition of amino acid prototrophy on minimal media, and streptomycin resistance. The number of E. coli CSH62 HfrH donor cells was determined by plating on minimal medium.

E.o.p. of phage P1 and λ Fec− mutants. The E.o.p. of phages P1 and λ Fec− was tested on E. coli strains RR1 (recA¹), HB101 (recA) and HB101(pRSR100). Cells were grown in Luria broth to an OD₆₀₀ of 0.2, harvested by centrifugation and resuspended in SM buffer (Maniatis et al., 1982). The cells (0.1 ml) were mixed with phage and adsorption was allowed to proceed for 20 min at room temperature. Agar (3 ml, 0.8%, w/v, 45 °C) was added, mixed and poured onto Luria agar plates. The number of plaques was determined after 18 h incubation at 37 °C.

Western blotting of proteins. Western blotting from SDS-PAGE gels onto nitrocellulose membranes was done by the method of Towbin et al. (1979). The membrane was blocked overnight at room temperature with 10 mM-Tris/HCl (pH 7.4), 0.15 M-NaCl, 2% (w/v) nonfat dried milk (Johnson et al., 1984) and 0.05% (v/v) Tween-20. The antibody binding and the development of bands using a goat anti-rabbit serum conjugated to horseradish peroxidase was done according to the method of Rybicki & von Wechmar (1982), except that Tween-20 was used instead of NP-40.

RESULTS

Isolation of the T. ferrooxidans recA gene. Plasmid DNA from a library of approximately 8000 colonies was prepared and transformed into E. coli HB101. Transformants were screened on ampicillin Luria agar plates containing 0.01% (w/v) MMS. Three different ampicillin- and MMS-resistant colonies were isolated. Plasmid DNA prepared from these colonies was used to retransform E. coli HB101. The recombinant plasmids were designated pRSR100, pRSR101 and pRSR102.
Cloning *T. ferrooxidans* recA gene

**Restriction mapping and DNA homology.** The recombinant plasmids pRSR100, pRSR101 and pRSR102 were physically mapped using several restriction endonucleases (Fig. 1). The sizes of the cloned fragments were 4.5, 4.0 and 3.4 kb in pRSR100, pRSR101 and pRSR102 respectively. Based on the restriction maps, the recombinant plasmids shared a common fragment of cloned DNA and therefore represented independent cloning events of the recA-like gene. EcoRV and EcoRI endonuclease deletions of pRSR100 and a BglII/BamHI endonuclease deletion of pRSR102 were constructed (Fig. 1). Only the EcoRI endonuclease deletion of pRSR100 still conferred resistance to MMS.

Southern blotting and DNA hybridization were used to confirm that the 4.5 kb DNA insert of pRSR100 originated from the *T. ferrooxidans* chromosome. Chromosomal DNA and pRSR100 were both digested with *PstI* endonuclease. The restriction enzyme fragments were separated on an agarose gel, Southern blotted and hybridized to 32P-labelled pRSR100. A positive hybridization signal was obtained with fragments of the *T. ferrooxidans* chromosomal digest corresponding exactly to fragments of pRSR100 produced from the *PstI* restriction sites internal to the 4.5 kb insert.

**UV, MMS and NQO sensitivity.** The effect of pRSR100 on the UV, MMS and NQO sensitivity of *E. coli* HB101 (recA) was determined by comparison with untransformed strain HB101 and the parental strain RR1 (recA+). The UV sensitivity of strain HB101(pRSR100) was intermediate between those of strains HB101 (recA) and the RR1 (rec+) (Fig. 2). *E. coli* HB101 carrying any of the three recombinant plasmids pRSR100, pRSR101 or pRSR102 was resistant to the same levels of MMS and NQO as *E. coli* RR1 (recA+). For example *E. coli* HB101(pRSR100) grew at concentrations of 0.1% (w/v) MMS and 10 μg NQO ml⁻¹, compared with maximum levels of 0.005% (w/v) MMS and 1 μg NQO ml⁻¹ for the untransformed strain.

**Recombination proficiency.** The ability of *E. coli* HB101(pRSR100) to carry out homologous recombination was compared with that of *E. coli* HB101 (recA) and the parental strain RR1 (recA+). The level of homologous recombination in *E. coli* HB101 was increased >10²-fold by pRSR100, although this value was only 15% of that of the recA+ strain RR1 (Table 2).
Fig. 2. Effect of the recombinant plasmid pRSR100 on the survival of UV-irradiated *E. coli* HB101. Cells were grown in Luria broth to an OD$_{600}$ of 0.2, collected by centrifugation and resuspended in an equal volume of M9 medium. The suspension was irradiated with increasing doses of 254 nm radiation at a dose rate of 1 J m$^{-2}$ s$^{-1}$. Survivors were determined on Luria agar after 18 h. ●, *E. coli* HB101 (recA); ■, *E. coli* HB101(pRSR100); ▲, *E. coli* RR1 (recA$^+$). The results are means of three experiments.

Table 2. Recombinational proficiency of pRSR100 as determined by genetic recombination following Hfr mating, and the e.o.p. of phages P1 and λ Fec$^-$

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Recombinational proficiency$^*$</th>
<th>Phage P1</th>
<th>λ Fec$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR1 (recA$^+$)</td>
<td>2.20</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>HB101(pRSR100)</td>
<td>0.34</td>
<td>0.79</td>
<td>0.21</td>
</tr>
<tr>
<td>HB101 (recA)</td>
<td>$2 \times 10^{-4}$</td>
<td>$&lt;1 \times 10^{-5}$</td>
<td>$&lt;1 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

$^*$ No. of transconjugants per 100 donor cells.

Replication of phages P1 and λ Fec$^-$: Strains of *E. coli* containing recA mutations are unable to support the growth of phage P1 (Cohen, 1983) or λ Fec$^-$ mutants, which carry the red and gam mutations (Smith, 1983). The e.o.p. of phages P1 and λ Fec$^-$ on *E. coli* HB101, HB101(pRSR100) and RR1 is shown in Table 2. The cloned gene restored the e.o.p. of phage P1 to levels close to those of the parental recA$^+$ strain. The λ Fec$^-$ mutant produced large plaques with an e.o.p. of 1.0 on *E. coli* RR1, but no plaques were observed on *E. coli* HB101. The ability of the λ Fec$^-$ mutant to grow on *E. coli* HB101 was restored by pRSR100, but the e.o.p. on *E. coli* HB101(pRSR100) was only 0.21 and the individual plaques were reduced to pin-pricks in size.

Western blotting. Rabbit antiserum prepared against *E. coli* RecA protein (Goodman et al., 1987) was used in Western blots to challenge proteins from crude extracts of *E. coli* strains HB101, HB101(pRSR100), JK696 and JK696(pRSR100). The *E. coli* recA gene product is a single polypeptide with an $M_r$ of approximately 40000. Previously we showed that the antiserum reacted with a polypeptide with an apparent $M_r$ of approximately 40000 in uninduced extracts of *E. coli* C600 cells; the production of this polypeptide was markedly increased after UV induction of *E. coli* C600 (Goodman et al., 1987). The antiserum reacted with a polypeptide of apparent $M_r$ approximately 40000 in cell extracts of uninduced *E. coli* HB101, which contains a mutation within the recA gene. This polypeptide was not induced by UV radiation (Goodman et
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No antiserum reaction was detected with cell extracts from the *E. coli* recA deletion mutant JK696. However, protein extracts from *E. coli* JK696(pRSR100) cells did react with the antiserum, and two protein bands, of apparent *M*, approximately 40000 and 38000, were detected. In *E. coli* HB101(pRSR100) cell extracts the antiserum reacted very strongly with a protein band of apparent *M*, approximately 40000 and with an additional protein band of apparent *M*, approximately 38000. Although it is difficult to obtain high concentrations of *T. ferrooxidans* cells, a faint band with an apparent *M*, of approximately 40000 was detected in *T. ferrooxidans* cell extracts with the antiserum.

**DISCUSSION**

This account of the cloning and characterization of the recA gene from *T. ferrooxidans* is apparently the first report of the presence or isolation of a recA gene from an autotrophic, chemolithotrophic bacterium. The cloned gene allows functional complementation of recombinase activity in an *E. coli* recA mutant. Evidence for this is the increase in the number of recombinants obtained by homologous recombination after Hfr-mediated conjugation, the growth of phage P1, which requires recombination between terminal repeats for replication and the suppression of the Fec− phenotype of phage λ. In each case the level of recombinase activity that resulted from the cloned *T. ferrooxidans* recA gene was considerably less than that of the recA+ E. coli strain R1.

Induction of DNA repair as a result of UV irradiation, MMS or NQO damage was also partially restored to the *E. coli* recA mutant by the cloned *T. ferrooxidans* gene. Since cleavage of the *lexA* repressor by the *recA* gene product is required to induce the SOS response, this indicates protease activity of the cloned gene product.

The observation that the product of the cloned *T. ferrooxidans* recA gene complemented the induction of the SOS response more completely than recombinase activity may indicate that the domains associated with protease activity are more functional in *E. coli* than the domains associated with recombinase activity.

It is likely that the cloned *T. ferrooxidans* recA gene product is expressed in *E. coli* from its own promoter. Deletions on either side of the cloned *T. ferrooxidans* chromosomal fragment or removal of the λ rightward promoter of the EcoRI structural gene of the pEcoR251 vector (the EcoRI deletion of pRSR100, Fig. 1) did not affect resistance to MMS. Further evidence for the expression of the *T. ferrooxidans* recA gene in *E. coli* from its own promoter was obtained by the reisolation and expression of the recA gene in both orientations from a cosmid library (unpublished results).

The cloned *T. ferrooxidans* recA-like gene produced two polypeptide bands, of apparent *M*, approximately 40000 and 38000, in an *E. coli* recA deletion mutant. Since a single polypeptide band of apparent *M*, approximately 40000 was detected in *T. ferrooxidans* it is suggested that the 38000 *M*, polypeptide detected in *E. coli*(pRSR100) is a degradation product of the 40000 *M*, polypeptide. Since the defective *E. coli* RecA protein in *E. coli* HB101 also has an *M*, of 40000 it was not possible to determine whether the cloned *T. ferrooxidans* protein enhanced the production of the native defective *E. coli* RecA protein after UV irradiation of *E. coli*(pRSR100) cells.

The presence of a recA-like gene in *T. ferrooxidans* indicates that the bacterium appears to have a homologous genetic recombination system. The implication of this finding for the genetic manipulation of *T. ferrooxidans* is that once a means of reintroducing DNA into the bacterium is discovered, it should be possible to recombine manipulated genes back into the chromosome.

**REFERENCES**


