Plasmid R46-mediated Protection Against Bleomycin is *polA*⁺-dependent

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Strains of *Escherichia coli* deficient in post-replication recombination repair were more sensitive to bleomycin than wild-type, repair-proficient strains. Mutants lacking excision repair functions were no more sensitive to bleomycin than the wild-type strains, indicating that this pathway is not involved in the repair of bleomycin-damaged DNA. Plasmid R46 not only protected repair-proficient strains but also those with *recB*, *recC*, *uvrA* or *lig* genotypes, suggesting that R46 protection against bleomycin is independent of these host repair functions. However, R46 protection was abolished in *recA* or *polA* strains, indicating that these gene functions are necessary for plasmid-mediated protection. It is suggested that protection may be due to a *recA*⁺-dependent interaction of a plasmid-encoded product with host DNA polymerase I, resulting in an increase in the DNA repair capacity of cells.

INTRODUCTION

Some R plasmids not only confer resistance to antibiotics but also protect their hosts against the lethal effects of ultraviolet (u.v.) light (Drabble & Stocker, 1968). A number of these plasmids also increase the survival of cells following DNA damage mediated by ionizing radiations or alkylating agents (MacPhee, 1972; Lehrbach *et al.*, 1977). The N group R plasmid R46 is amongst those known to protect against u.v. light (Drabble & Stocker, 1968). This protective effect has been shown to be completely dependent upon the *recA*⁺ genotype in *Salmonella typhimurium*, but independent of the *uvrB* or *polA* gene functions (Mortelmans & Stocker, 1976). In *Escherichia coli*, R46 does not protect *recA* mutants but does protect *recB*, *recC*, *uvrA*, *polA* or *lig* mutants (Tweats *et al.*, 1976). It is therefore thought that u.v.-protecting plasmids function by interacting with *recA*⁺-dependent, error-prone (SOS) repair processes (Walker, 1977).

Bleomycin (BLM) is an anticancer drug that binds to DNA causing release of free bases (Haidle *et al.*, 1972) and single- and double-strand scissions (Suzuki *et al.*, 1969; Lloyd *et al.*, 1978). DNA strand cleavage by BLM may require Fe²⁺ (Sausville *et al.*, 1976) and recent studies have suggested interaction of the drug with specific base sequences (D'Andrea & Haseltine, 1978; Takeshita *et al.*, 1978). The various effects of BLM on DNA structure make it a useful agent for studying the ability of certain plasmids to protect their hosts against DNA damage. Experiments were therefore designed to test the effect of BLM on *E. coli* strains that are either repair-proficient or repair-deficient and harbour the u.v.-protecting plasmid R46.

METHODS

Chemicals. Bleomycin (batch no. 211) was a generous gift from Lundbeck Ltd; it was dissolved at 1 mg ml⁻¹ in sterile distilled water and stored at 4 °C. Calf thymus DNA was purchased from Sigma. Non-radioactive deoxyadenosine 5'-triphosphate (dATP), deoxycytidine 5'-triphosphate (dCTP), deoxyguanosine 5'-triphosphate

Table 1. *Strains of Escherichia coli K12 used*

Strain	Genotype	Source	Reference
AB1157	F ⁻ <i>thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 strA31 sup-37</i>	N. Willetts	Howard-Flanders <i>et al.</i> (1964)
AB2463	As AB1157 but also <i>recA13</i>	N. Willetts	Howard-Flanders <i>et al.</i> (1966)
AB2470	As AB1157 but also <i>recB21</i>	N. Willetts	Howard-Flanders <i>et al.</i> (1966)
JC5495	As AB1157 but also <i>recA13 recB21</i>	N. Willetts	Willetts & Clark (1969)
NH4021	<i>met arg thi gal tsx recC22</i>	P. Barth	—
AB1886	As AB1157 but also <i>uvrA6</i>	N. Willetts	Boyce & Howard-Flanders (1964)
N1325	<i>thi trp bio lac gal ara tsx lop-8 lig-2</i>	M. Gellert	Gellert & Bullock (1970)
MM300	<i>ilv thyA rha lacZ str</i>	M. Monk	Monk & Kinross (1972)
MM386	<i>rha lacZ str polA12</i>	M. Monk	Monk & Kinross (1972)
JG138	<i>thyA rha lacZ str polA1</i>	M. Monk	Monk <i>et al.</i> (1971)
AB2545	<i>thi-1 metE46</i>	B. Bachmann	Eggertsson & Adelberg (1965)
AB2545-1	<i>thi-1 polA1</i>	This paper	—

(dGTP) and thymidine 5'-triphosphate (dTTP) were obtained from P-L Biochemicals Ltd. [*meth*yl-³H]Thymidine 5'-triphosphate was supplied by Amersham.

Bacterial strains and culture conditions. All strains used were derivatives of *Escherichia coli* K12 and are listed with their genotypes in Table 1. Plasmid R46, which confers resistance to ampicillin, streptomycin, sulphonamides and tetracycline, was transferred into these strains by conjugation from *E. coli* strain J5-3 *pro met* (R46) using the method described by Smith (1969).

Cultures were grown routinely in Oxoid nutrient broth no. 2 (code CM67). Solid media were nutrient agar, which was Oxoid blood agar base (code CM55), or Oxoid MacConkey agar (code CM7). Soft agar overlays contained (per 100 ml distilled water): 2.25 g nutrient broth, 0.35 g Oxoid Ionagar (code L12) and 1 mM-CaCl₂. Davis & Mingioli (DM) liquid and solid media were prepared as described by Smith (1967).

Survival of cells exposed to BLM. Overnight nutrient broth cultures of test strains were diluted into fresh nutrient broth containing BLM. At the indicated times BLM-treated cells were diluted in nutrient broth, plated on MacConkey agar and incubated overnight to determine survival. The temperature-sensitive mutant *E. coli* MM386 *polA12* was grown, tested and counted, either at 30 °C (permissive temperature) or at 42 °C (restrictive temperature). All other strains were grown, tested and counted at 37 °C.

Preparation of phage P1 lysate and transduction of the *polA1* allele. The structural gene for DNA polymerase I (*polA*) lies midway between the *metE* and *rha* loci on the *E. coli* K12 chromosome (Gross & Gross, 1969). Therefore it is possible to cotransduce the *polA* gene with either of these markers by use of a generalized transducing phage such as P1. A suspension of phage P1CM *clr100* was prepared by thermal induction of *E. coli* strain N99 (Rosner, 1972). The phage suspension was filter-sterilized through a 0.45 µm membrane filter (Millipore) and a stock of transducing phage of known titre was prepared on *E. coli* JG138 *polA1 metE*⁺ by the following procedure. A 10-fold dilution series of P1CM was prepared in nutrient broth and 0.1 ml of each dilution was mixed, in 2.5 ml soft agar, with 0.1 ml of an exponential phase bacterial culture. The soft agar overlays were then poured on to nutrient agar plates and incubated overnight at 37 °C. Phage were harvested from plates showing semi-confluent lysis by scraping off the soft agar and vortex mixing with 2.5 ml nutrient broth per overlay. After centrifugation at 6000 g for 30 min, the resultant transducing phage suspension was filter-sterilized. A 50 ml exponential phase culture of strain AB2545 *polA*⁺ *metE* was washed and resuspended in 5 ml nutrient broth containing 1 mM-CaCl₂. To 1 ml portions of this bacterial suspension were added 1 ml portions of the P1CM suspension, isolated from *E. coli* JG138 *polA1 metE*⁺, to give multiplicities of infection of between 0.1 and 10.0 phage per bacterial cell. Phage were allowed to adsorb for 30 min at 30 °C and the suspension was then washed twice in DM salts before plating on solid DM medium that selected for Met⁺ transductants of *E. coli* AB2545. Colonies appeared after 48 h incubation at 37 °C and were streaked on to DM medium without methionine. Fifty Met⁺ clones were then tested for sensitivity to u.v.-irradiation to check for the presence of the *polA1* mutation.

U.v. light sensitivity testing of *metE*⁺ transductants. Overnight cultures of *metE*⁺ transductants were diluted in nutrient broth in a 10-fold series to 10⁻⁶ and 0.02 ml drops of each dilution were placed on nutrient agar plates. The drops were allowed to absorb into the agar at room temperature before exposing the plates to u.v. light from a Hanovia model 12 low pressure mercury lamp that emitted light at 254 nm. After overnight incubation at 37 °C, the u.v. light sensitivity of the *metE*⁺ transductants was compared with that of *E. coli* JG138 *polA1* and *E. coli* AB2545 *polA*⁺. Clones showing u.v. sensitivity comparable to that of strain JG138 *polA1* were further tested for the presence of the *polA1* mutation by direct measurement of their DNA polymerase I activity *in vitro*.

Measurement of DNA polymerase I activity of u.v. light-sensitive *metE*⁺ transductants. Overnight, 100 ml nutrient broth cultures of *E. coli* JG138 *polA1*, *E. coli* AB2545 *polA*⁺ *metE* and u.v. light-sensitive *metE*⁺

transductants of strain AB2545 were harvested by centrifugation for 30 min at 4 °C and 6000 g. Cells were resuspended in 5 ml cold 0.1 M-Tris buffer, pH 7.4 containing 0.01 M-MgSO₄. Suspensions were disrupted using an MSE 150 W ultrasonic disintegrator for a total of 90 s at a peak amplitude of 6 µm. The extracts were then centrifuged for 20 min at 4 °C and 12 500 g and the supernatants were decanted and placed on ice.

DNA polymerase I activity was assayed in reaction mixtures (250 µl) that contained 50 mM-Tris pH 7.4, 10 mM-MgSO₄, 5 µg calf thymus DNA which had been fragmented by ultrasonic disintegration for 90 s, 20 µM each of dATP, dCTP and dGTP, 2 µM-dTTP, 2.5 µCi [³H]dTTP ml⁻¹ (50 Ci mmol⁻¹, 1.85 TBq mmol⁻¹) and 100 µl cell extract. Reaction mixtures were incubated at 30 °C and 50 µl samples were removed at intervals, spotted on to Whatman 3MM chromatography paper and washed three times with ice-cold 5% (w/v) trichloroacetic acid/0.1% (w/v) tetra-sodium pyrophosphate. They were then washed in absolute alcohol, rinsed in ether, dried and counted in a Packard Tri-carb liquid scintillation counter, using a toluene-based scintillant.

RESULTS

R46-mediated protection against BLM in DNA repair-proficient E. coli strains

Plasmid R46 conferred protection against BLM on *E. coli* AB1157 which is wild-type with respect to DNA repair. Protection was apparent at drug concentrations that were lethal or sub-lethal to R⁻ bacteria (Fig. 1).

Effect of R46 on survival of post-replication repair-deficient E. coli strains

Escherichia coli strains AB2470 *recB* and NH4021 *recC* lack ATP-dependent exonuclease V activity and are therefore deficient in recombination processes including post-replication recombination repair (Clark, 1973). Strain AB2463 *recA* is deficient in the *recA*-coded protein X, which controls exonuclease V activity (Gudas & Pardee, 1975), and the inducible, *recA*⁺-dependent SOS repair processes (Witkin, 1976; Devoret, 1978). Strains that are *recA* therefore lack both post-replication recombination repair and SOS repair pathways. The *recA* mutant was more sensitive to BLM than the wild-type strain AB1157, and whereas R46 increased the survival of the repair-proficient strain, it failed to protect the *recA* mutant to any significant extent (Table 2). Hence R46-mediated BLM protection is *recA*⁺-dependent. Results obtained with all the post-replication repair-deficient strains are summarized in Table 2. All of these strains were more sensitive to BLM than *E. coli* AB1157. Moreover, although R46 failed to increase to any marked degree the survival of strains that are *recA* or *recA recB*, it did significantly protect the *recB* and *recC* mutants, indicating that protection against BLM

Fig. 1. Plasmid R46-mediated protection against BLM in the repair-proficient *E. coli* strain AB1157. Cells were incubated in nutrient broth containing 1 µg BLM ml⁻¹ [AB1157 (○), AB1157 (R46) (●)], or 0.1 µg BLM ml⁻¹ [AB1157 (□), AB1157 (R46) (■)], or without BLM [AB1157 (Δ), AB1157 (R46) (▲)]. At hourly intervals samples were diluted in nutrient broth and plated on MacConkey agar. Survival was determined after overnight incubation at 37 °C.

Table 2. *Effect of plasmid R46 on survival of post-replication repair-deficient E. coli strains exposed to BLM*

Cells (10^7 ml $^{-1}$) were incubated at 37 °C in nutrient broth containing 1 µg BLM ml $^{-1}$. After 1 h, samples were diluted in nutrient broth and plated on MacConkey agar to test for viability. Percentage survival refers to the numbers of survivors after 1 h in BLM compared with untreated cultures.

Strain	Relevant genotype	Missing repair function	Percentage survival	
			R $^-$	R46
AB1157	Wild-type	None	30	60
AB2463	<i>recA</i>	Protein X	1.5	2
JC5495	<i>recA recB</i>	Protein X and exonuclease V	10	12
AB2470	<i>recB</i>	Exonuclease V	4	12
NH4021	<i>recC</i>	Exonuclease V	9	30

Table 3. *Effect of plasmid R46 on survival of excision repair-deficient E. coli strains exposed to BLM*

Cells were exposed to 1 µg BLM ml $^{-1}$ (except where indicated) for 1 h, as described in Table 2.

Strain	Relevant genotype	Missing repair function	Percentage survival	
			R $^-$	R46
AB1157	Wild-type	None	30	60
AB1886	<i>uvrA</i>	Incision endonuclease	36	68
N1325	<i>lop lig</i>	DNA ligase (defective)	34	75
JG138	<i>polA1</i>	DNA polymerase I	75	80
JG138*	<i>polA1</i>	DNA polymerase I	15	17
MM300	Wild-type	None	25	57

* Cells were exposed to 3 µg BLM ml $^{-1}$.

is independent of the *recB* $^+$ and *recC* $^+$ genotypes. These results are comparable to those observed for R46 when protecting against u.v. light (Tweats *et al.*, 1976).

Effect of R46 on survival of excision repair-deficient E. coli strains

Escherichia coli AB1886 *uvrA* lacks the ATP-dependent endonuclease activity required for initiation of the excision repair pathway (Braun & Grossman, 1974). *Escherichia coli* strain N1325 *lop lig* produces a defective DNA ligase and this renders it deficient in the final resealing step of excision repair (Hanawalt, 1968). Each of these mutants had a similar sensitivity to BLM when compared with the wild-type strain, *E. coli* AB1157 (Table 3). Furthermore, R46 protected both the *uvrA* mutant and the *lop lig* mutant to the same extent as the repair-proficient strain (Table 3). This indicates that plasmid-mediated protection against BLM is independent of the *uvrA* $^+$ or *lig* $^+$ genotypes, which again agrees with results for R46 protection against u.v. irradiation (Tweats *et al.*, 1976).

Escherichia coli JG138 *polA1* is deficient in the DNA polymerizing function of DNA polymerase I but possesses normal levels of the 5' to 3' exonuclease activity associated with this enzyme (De Lucia & Cairns, 1969; Lehman & Chien, 1973). Hence, this mutant is unable to perform the normal DNA synthesis step in excision repair. Surprisingly, strain JG138 was more resistant to BLM than the repair-proficient strains AB1157 and MM300, and R46-mediated protection was not apparent in the *polA1* mutant (Table 3). The relative resistance to BLM of *E. coli* JG138 *polA1* suggested that the drug was inducing lower levels

Fig. 2

Fig. 3

Fig. 2. Lack of plasmid R46-mediated protection against BLM in *E. coli* strain AB2545-1 *polA1*. Cells were exposed to various concentrations of BLM for 1 h, as described in Table 2. AB2545-1 *polA1* (○), AB2545-1 *polA1* (R46) (●), AB2545 *polA*⁺ (□), AB2545 *polA*⁺ (R46) (■).

Fig. 3. Plasmid R46-mediated protection against BLM in *E. coli* strain MM386 *polA12* at the permissive temperature but not at the restrictive temperature. Cells (10^7 ml⁻¹) were grown overnight at 30 °C (permissive temperature) or 42 °C (restrictive temperature) in nutrient broth. They were then subcultured into nutrient broth containing BLM and after 1 h were diluted in nutrient broth, plated on MacConkey agar and incubated at either 30 °C or 42 °C, respectively, to determine viability. MM386 *polA12* at 42 °C (○), MM386 *polA12* (R46) at 42 °C (●), MM386 *polA12*⁺ at 30 °C (□), MM386 *polA12*⁺ (R46) at 30 °C (■).

of DNA damage in this mutant than in the wild-type (*polA*⁺) strains and it was plausible that this could account for the failure to detect R46-mediated protection in the *polA1* mutant. However, the plasmid still failed to protect JG138 *polA1* following treatment with a concentration of BLM (3 μg ml⁻¹) that resulted in lower levels of survival, at which protection was observed in wild-type and other excision repair-deficient strains (Table 3). This suggests that R46-mediated protection against BLM is *polA*⁺-dependent, which is in contrast to plasmid-mediated protection against u.v. light (Tweats *et al.*, 1976).

Effect of the *polA* mutation on R46-mediated protection

Other workers have shown *polA*⁺ and *polA*⁻ strains of *E. coli* to have similar sensitivities to BLM (Ross & Moses, 1976; Yamamoto & Hutchinson, 1979). Therefore, the finding that *E. coli* JG138 *polA1* was more resistant to BLM than *polA*⁺ strains, and that R46 protection was *polA*⁺-dependent, required further investigation.

Our approach was to transduce the *polA1* allele from strain JG138 *polA1 metE*⁺ into a new host, *E. coli* AB2545 *polA*⁺ *metE*, using phage P1. In order to identify *polA1* derivatives, the *metE*⁺ transductants of strain AB2545 were tested for their sensitivity to u.v. light and for their ability to synthesize DNA (in the absence of ATP) *in vitro*. Using these criteria it was found that the *polA1* allele had cotransduced with the *metE*⁺ gene function at a frequency of 12%. This result was in close agreement with the cotransduction frequency of 17% reported by Gross & Gross (1969). A newly constructed *polA1 metE*⁺ mutant, designated AB2545-1, showed the same sensitivity to u.v. light as *E. coli* strain JG138 *polA1* (data not shown). Moreover, *E. coli* AB2545-1 *polA1* possessed less than 1% DNA polymerase I activity *in vitro* when compared with the *polA*⁺ strain *E. coli* AB2545 (data not shown). Strain AB2545-1 *polA1* was found to have a sensitivity to BLM similar to that of *E. coli* AB2545 *polA*⁺ (Fig. 2). However, although strain AB2545 *polA*⁺ was protected by R46, the

Table 4. *Effect of plasmid R46 on survival of E. coli polA strains exposed to BLM*Cells were exposed to 1 µg BLM ml⁻¹ for 1 h, as described in Table 2.

Strain	<i>polA</i> characteristic	Percentage survival	
		R ⁻	R46
AB2545	Wild-type	26	57
AB2545-1	<i>polA1</i>	20	23
MM300	Wild-type	25	57
JG138	<i>polA1</i>	75	80
MM386 (30 °C)	<i>polA12</i> ⁺	12	54
MM386 (42 °C)	<i>polA12</i>	9	10

plasmid did not increase the survival of strain AB2545-1 *polA1* (Fig. 2), which again implies that R46 protection against BLM is *polA*⁺-dependent.

Further evidence for the *polA*⁺-dependence of R46 protection against BLM came from work with *E. coli* strain MM386. This strain carries the temperature-sensitive *polA12* mutation which results in loss of the 5' to 3' exonuclease, 3' to 5' exonuclease and polymerizing functions of DNA polymerase I at restrictive temperatures (Uyemura & Lehman, 1976). There was little difference in the sensitivity to BLM of *E. coli* MM386 at permissive or restrictive temperatures, but whilst R46 protected the strain at the permissive temperature it failed to do so at the restrictive temperature (Fig. 3).

A summary of results given by the various *polA* mutants (Table 4) shows that, of these strains, only JG138 *polA1* was more resistant to BLM than the *polA*⁺ strain. However, loss of R46-mediated protection against BLM was apparent in all of those *polA* mutants tested, indicating that the protective effect is indeed *polA*⁺-dependent.

DISCUSSION

Strains of *E. coli* that are deficient in post-replication repair were more sensitive to BLM than post-replication repair-proficient strains (Table 2). The excision repair-deficient strains were no more sensitive to BLM than strains that are proficient in this repair pathway (Table 3). In fact, *E. coli* JG138 *polA1* was more resistant to BLM than *polA*⁺ strains (Table 3). However, other *polA* mutants were no more resistant to BLM than wild-type *E. coli* (Table 4), which suggests that the BLM resistance of strain JG138 *polA1* is due to some factor(s) other than DNA polymerase I deficiency. For example, it is possible that this strain has reduced permeability to BLM. Indeed, Yamagami *et al.* (1974) have shown that such a permeability barrier exists and that it is under the control of a gene (designated *blm*) located in the membrane cluster region.

Our finding that *E. coli* strains deficient in excision repair functions are no more sensitive to BLM than repair-proficient strains is in disagreement with that recently reported by Ross *et al.* (1980), who found excision repair-deficient mutants to have increased sensitivity to BLM. However, other workers have shown that whereas strains of *E. coli* lacking post-replication repair are more sensitive to BLM than wild-type strains, mutants deficient in excision repair functions are no more sensitive to the drug than repair-proficient strains (Yamagami *et al.*, 1974; Otsuji *et al.*, 1978; Yamamoto & Hutchinson, 1979). Furthermore, the phleomycins, which show a close structural similarity to BLM (Takita *et al.*, 1972) and also induce strand scission in DNA (Grigg, 1969; Farrel & Reiter, 1973), are more lethal to *recA*, *recB* or *recA recB* double mutants than to wild-type strains, whereas mutants that are *uvrA*, *uvrB*, *uvrC*, *uvrD* or *polA* are no more sensitive to the drug (Nakayama, 1975) than are wild-type strains. Hence, the repair of DNA strand breaks mediated by BLM or phleomycins would seem to involve post-replication repair processes rather than excision repair functions.

Plasmid R46 protected repair-proficient *E. coli* against BLM (Fig. 1). The plasmid could either code for a repair pathway that is separate from host repair processes, or could interact with host repair functions. Protection was dependent upon the host *recA*⁺ genotype but was independent of the *recB*⁺ or *recC*⁺ genotypes (Table 2), which suggests that the plasmid interacts with *recA*⁺-dependent SOS repair (Witkin, 1976; Devoret, 1978) rather than recombination repair, which involves the *recB recC*-encoded exonuclease. R46-mediated protection against BLM is *polA*⁺-dependent (Figs 2 and 3; Table 3 and 4) and this indicates that DNA polymerase I is involved in plasmid-mediated protection against the drug. This is somewhat surprising since DNA polymerase I does not normally participate in repair of BLM-damaged DNA (Table 4) and R46-mediated protection against u.v. light is independent of the *polA*⁺ function (Mortelmans & Stocker, 1976; Tweats *et al.*, 1976). However, it is plausible that a *recA*⁺-dependent interaction between an R46-mediated gene product and host DNA polymerase I could increase the BLM-damaged DNA repair capacity of cells by functioning in addition to pathways normally involved in repair of BLM-mediated DNA damage.

Treatment of DNA with BLM releases free nitrogenous bases (Haidle *et al.*, 1972) to leave apurinic and apyrimidinic sites (Povirk *et al.*, 1977; Ross & Moses, 1978). BLM also cleaves the deoxyribose-phosphate backbone of DNA (Lloyd *et al.*, 1978) and this results in formation of a variety of termini at gaps in the DNA structure (Kuo & Haidle, 1973). Takeshita *et al.* (1978) have suggested that BLM-mediated cleavage of the 3' to 4' carbon bond in the deoxyribose ring releases a malonic aldehyde-like product leaving a 3'-terminus consisting of a two-carbon fragment esterified to a phosphate group. DNA polymerase I of *E. coli* requires a free 3'-hydroxyl group at the primer terminus for either polymerization or for 3' to 5' exonucleolytic cleavage to occur (Huberman & Kornberg, 1970). Hence, at least some types of BLM damage do not serve as suitable primer termini for DNA polymerase I activity. Indeed, Niwa & Moses (1981) have shown that BLM-damaged DNA is not a suitable substrate for purified DNA polymerase I activity *in vitro*. However, purified *E. coli* exonuclease III is able to convert BLM-damaged DNA into a suitable template-primer for polymerization (Niwa & Moses, 1981). It is possible, therefore, that a plasmid-encoded product, such as a nuclease, could modify the termini of BLM-damaged DNA in preparation for DNA polymerase I repair activity. Such an interaction between plasmid and host repair functions would be expected to amplify the DNA repair capacity of cells and therefore increase their level of survival.

The mechanism by which some R plasmids protect bacteria against the lethal effects of DNA damage remains unclear. However, this study suggests that DNA damaging drugs such as BLM may be useful tools in elucidating the nature of interactions between plasmid and host repair processes.

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