Photochemistry and Photobiology, 1974, Vol. 20, pp. 9–13. Pergamon Press. Printed in Great Britain

SENSITIZATION OF ULTRAVIOLET-IRRADIATED *Escherichia coli* K–12 BY DIFFERENT AGARS: INHIBITION OF A *rec* AND *exr* GENE-DEPENDENT BRANCH OF THE *uvr* GENE-DEPENDENT EXCISION-REPAIR PROCESS

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(*Received* 20 *August* 1973; *accepted* 10 *December* 1973)

Abstract—*E. coli* K–12 wild-type cells plated immediately after UV irradiation had a much lower survival on minimal medium solidified with agar–agar No. 3 (Oxoid), purified agar (Difco) or ionagar (Colab) than on plates solidified with laboratory-washed Noble agar (Difco). An intermediate survival was obtained on plates solidified with unwashed Noble agar (Difco). When irradiated cells were incubated in liquid minimal medium for various times before subsequent plating on agar–agar No. 3, their survival increased rapidly and became identical to the survival of cells plated on washed Noble agar. The same phenomenon was found with *polA* 1 cells, but no differences in survival on the different agars were observed with *uvrB*, *exrA*, *recA* or *recB* cells. These data suggest that a repair process dependent on the *uvrB*, *recA*, *recB* and *exrA* (but not the *polA*) gene products is inhibited when UV irradiated *E. coli* K–12 cells are plated on minimal medium solidified with agar–agar No. 3, purified agar or ionagar. This implies that the *uvr* gene-dependent excision–repair process consists of at least two branches, one controlled by the *polA* gene and a second controlled by the *recA*, *recB* and *exrA* genes and inhibitable by a substance present in certain agars. For maximum sensitivity, experiments designed to study chemical inhibitors of repair should use agar plates that do not themselves inhibit repair.

INTRODUCTION

Various compounds have been shown to inhibit the repair of UV induced damage in *E. coli* cells. Acriflavine and caffeine (Witkin, 1969b for review) appear to inhibit both the post-replicational–repair and excision–repair processes. Chloramphenicol does not block excision of pyrimidine dimers (Swenson and Setlow, 1966) but does inhibit the repair of single-strand breaks produced in DNA by the excision–repair process (Youngs *et al.*, 1974). Chloramphenicol also decreases the extent of post-replicational repair.*

Reductone (Alcantara Gomes *et al.*, 1970), 2,4-dinitrophenol (DNP) (Van der Schueren and Smith, 1974), and probably also chloroquine (K. C. Smith, unpublished results) seem to inhibit only the excision–repair system. This is indicated by the observation that these compounds sensitize wild-type but not *uvr* strains to UV radiation. More specifically, DNP seems to inhibit a branch of the excision–repair process which is controlled by the *recA*, *recB* and *exrA* genes, since strains containing a *rec* or *exrA* mutation are not sensitized to UV radiation by DNP (Van der Schueren and Smith, 1974). The effect of reductone treatment on wild-type, *uvr* and *recA* strains after UV irradiation was the same as that of DNP treatment (L. R. Caldas, personal communication).

The present results indicate that several brands of agar contain an inhibitor of the *uvr* gene-dependent excision–repair process. The inhibitory effect appears to be specific for the branch of excision repair controlled by the *recA*, *recB* and *exrA* genes. The effect could be eliminated by extensive washing of Noble agar before use.

*Ganesan and Smith (1970) found that chloramphenicol had no effect on the gap-filling step of post-replicational repair after an incident dose of UV radiation of 6 J m^{-2}. However, subsequent studies have shown that this compound does inhibit post-replicational repair after incident UV doses of 20 J m^{-2} or greater. A *recB* mutation was found to have an effect similar to that of chloramphenicol (Youngs and Smith, unpublished results), consistent with the hypothesis of Ganesan and Smith (1970) that chloramphenicol inhibits a *recB* gene-dependent repair process.

MATERIALS AND METHODS

Bacterial strains. All strains used were derivatives of *E. coli* K–12. The strains and their characteristics are listed in Table 1.

Table 1. *Escherichia coli* K–12 derivatives used*

Designation	Relevant genotype	Other markers	Reference of source
AB2497	wild–type	*arg his leu pro thi thr thy ara gal lac mtl xyl tsx str*	Howard–Flanders and Boyce (1966)
AB2487	*recA* 13	*arg his leu pro thi thr thy ara gal lac mtl xyl tsx str*	Howard–Flanders and Boyce (1966)
SR78	*recB* 21	*arg his leu pro thi thr thy ara gal lac mtl xyl tsx str*	Kapp and Smith (1970)
AB2499	*uvrB* 5	*arg his leu pro thi thr thy ara gal lac mtl xyl tsx str*	Howard–Flanders *et al.* (1966)
JG138	*polA* 1	*thy rha lac str*	Monk *et al.* (1971)
DY95	*exrA*	*thy rha lac str*	D. A. Youngs

*Abbreviations are as used by Taylor (1970). All strains were of the F⁻ mating type.

Media. The cells were grown in supplemented minimal medium (SMM) (Ganesan and Smith, 1968) which contained thiamine hydrochloride at 0·5 μg/ml, the necessary amino acids at 10^{-3} *M*, and thymine at 10 μg/ml for overnight cultures or 2 μg/ml for exponentially growing cells. DTM buffer (Kaplan *et al.*, 1962) was SMM without glucose, thiamine or supplements.

Agar. The agars used were agar–agar No. 3 (Oxoid), ionagar (Colab), Noble agar (Difco) and purified agar (Difco). Washed Noble agar (Difco) was Noble agar that had been washed ten times in 5–10 volumes of double-distilled water by suspension and decantation, once with redistilled 95 per cent ethanol, once with acetone, and air dried.

Survival curves. Overnight cultures were diluted 1 : 100 in SMM and incubated at 37°C. Cells were grown for about three cell doublings to a density of ~ 10^8 cells/ml. These cells were considered to be in log-phase growth. Cells were collected on a 0·45 μm membrane filter (Millipore Corp.) and resuspended in DTM buffer to a density of about 2×10^8 cells/ml. The UV source was a General Electric germicidal lamp (8 W) emitting primarily at 254 nm with an incident dose rate of 967 mW m^{-2} as determined with an International Light germicidal photometer (No. IL-254). The incident dose rate was reduced with a perforated screen to 55 mW m^{-2} for the sensitive mutants. Samples of 10 ml were irradiated in an open Petri dish (diameter 9 cm) while shaking on a platform shaker. To prevent photoreactivation, the irradiation and handling of the cells were carried out under General Electric 'gold' fluorescent lights.

After irradiation the cells were diluted 1 : 1 into SMM with twice the required supplements and incubated at 37°C. After different periods of incubation, samples were diluted in phosphate buffer (11·7 g Na_2HPO_4, 7·1 g KH_2PO_4 per liter, pH 7·0) and plated on SMM (pH 6·5) solidified with different agars (1·6 per cent, except for agar–agar No. 3 which was 0·9 per cent).

RESULTS

The survival of *E. coli* K–12 wild–type cells exposed to 110 J m^{-2} of UV radiation (254 nm) and plated immediately on agar–agar No. 3 was about 27–fold lower compared to cells plated on washed Noble agar (Fig. 1A). When Noble agar was used without previous washing, an intermediate survival was obtained (the survival was 3–fold lower than on washed Noble agar).

When the UV irradiated wild–type cells were incubated in liquid SMM before plating, the survival on agar–agar No. 3 and Noble agar increased rapidly while the survival on washed Noble agar

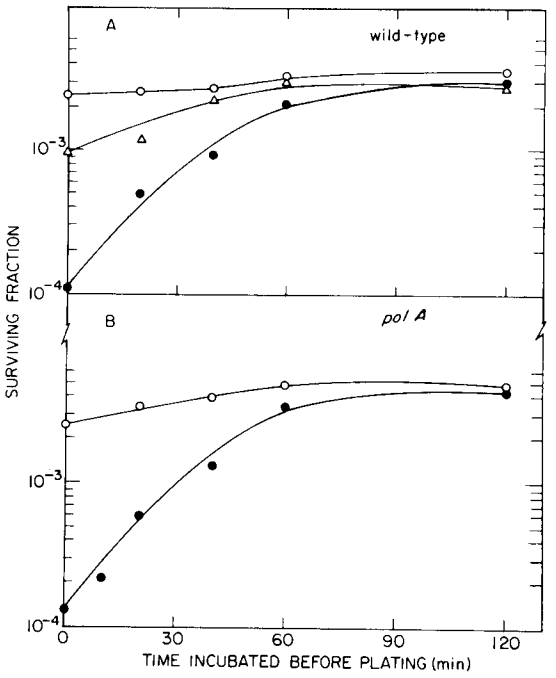

Figure 1. Influence of agar and incubation time prior to plating on the survival of UV irradiated cells. (A) wild type, AB2497; (b) *polA* 1, JG138. The incident doses of UV radiation were 110 J m^{-2} for AB2497 and 20 J m^{-2} for JG138. Cells were incubated in liquid SMM at 37°C and then plated on SMM solidified with washed Noble agar (○), Noble agar (△), or agar–agar No. 3 (●). For the wild–type cells the surviving fraction was calculated relative to an unirradiated sample plated at zero time on the same type of agar. With the *polA* 1 strain the plating efficiency of unirradiated cells on agar–agar No. 3 was about 44 per cent compared to cells plated on washed Noble agar. Thus, the surviving fraction for the *polA* 1 cells was calculated relative to unirradiated cells plated on washed Noble agar. The results shown are the average of three or more experiments per data point.

showed only a small increase during the 2 h incubation period. After 60 min of incubation the difference in survival on the three agars was less than a factor of 2, and after 120 min they were essentially identical (Fig. 1A).

A similar phenomenon was observed when *polA* 1 cells were studied (Fig. 1B). A lower survival was found on agar–agar No. 3 than on washed Noble agar when the cells were plated immediately after irradiation. Identical survivals were observed after incubation for 120 min in SMM before plating. For the *polA* 1 cells, a difference in colony-forming ability of the non-irradiated cells on the two agars was also found; colony-forming ability of unirradiated *polA* 1 cells was reduced to about 44 per cent when plated on agar–agar No. 3, compared with washed Noble agar. However, this is a small difference compared with that observed in UV irradiated cells.

An inhibitory effect on wild-type cells was also observed using purified agar (Difco) and ionagar (Colab). The surviving fraction increased by a factor of 7 for purified agar and 29 for ionagar when the cells were incubated for 60 min in liquid SMM at 37°C compared to samples plated immediately (Table 2).

A difference in survival of UV irradiated cells plated on agar–agar No. 3 vs washed Noble agar was not found for the *uvrB* (Fig. 2A), *recA* (Fig. 2B), *exrA* (Fig. 2C) or *recB* (Fig. 2D) strains. For UV irradiated cells of these strains, the surviving fractions on both agars were essentially equal and showed only a small increase during the incubation period in SMM.

No difference was found in the survival of X-irradiated wild-type and *polA* 1 cells on agar–agar No. 3 vs washed Noble agar (results not shown).

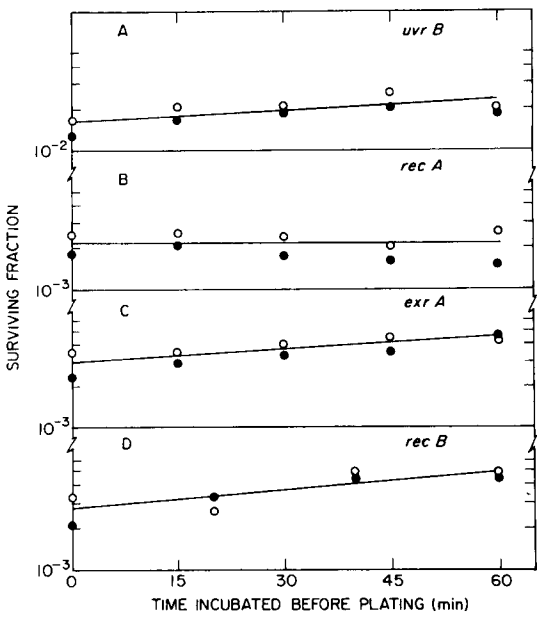

Figure 2. Influence of agar and incubation time prior to plating on the survival of UV irradiated cells. The strains and incident doses of UV radiation were (A) *uvrB*, AB2499, 12·5 J m^{-2}; (B) *recA*, AB2487, 2·5 J m^{-2}; (C) *exrA*, DY95, 12 J m^{-2}; (D) *recB*, SR78, 50 J m^{-2}. UV irradiated cells were incubated in liquid SMM at 37°C and then plated on SMM solidified with washed Noble agar (○) or agar–agar No. 3 (●). The results of a typical experiment with each strain are shown. The surviving fraction was calculated relative to an unirradiated sample plated at zero time on the same type of agar.

DISCUSSION

A much lower survival was observed when *E. coli* K–12 wild-type cells were plated immediately after UV irradiation on agar–agar No. 3 than on washed Noble agar (Table 2). This suggested that

Table 2. The surviving fraction of UV irradiated wild-type (AB2497) cells of *E. coli* K–12 relative to unirradiated controls plated at time zero

Agar	A Plated immediately after irradiation*	B Plated after 60 min incubation in SMM at 37°C*	Ratio B/A
Washed Noble agar (Difco)†	$2·5 \times 10^{-3}$	$3·4 \times 10^{-3}$	1·4
Noble agar (Difco)	$9·7 \times 10^{-4}$	$3·1 \times 10^{-3}$	3·2
Purified agar (Difco)	$4·7 \times 10^{-4}$	$3·3 \times 10^{-3}$	7·0
Agar–agar No. 3 (Oxoid)	$1·1 \times 10^{-4}$	$2·1 \times 10^{-3}$	19·1
Ionagar (Colab)	$9·2 \times 10^{-5}$	$2·7 \times 10^{-3}$	29·4

*Incident dose: 110 J m^{-2} at 254 nm. The results shown are the average of three or more experiments.

†Noble agar (Difco) was washed ten times in 5–10 volumes of double-distilled water by suspension and decantation, once with redistilled 95 per cent ethanol, once with acetone, and air dried.

some component of the agar–agar No. 3 inhibited a repair process acting on UV induced lesions. The *uvrB* strain, which is deficient in the incision step of the excision repair process (Howard–Flanders, 1968; Kato, 1972; Martignoni and Smith, 1973; Seeberg and Johansen, 1973), showed no difference in survival under these conditions. Thus, the effect on the survival of wild–type cells appears to be due to the presence in agar–agar No. 3 of an inhibitor of the *uvr* gene-dependent excision–repair process.

It appears that once an incision break has been made in UV irradiated DNA by the *uvr* gene products, several pathways exist for subsequent steps of the excision–repair process. Survival data (Monk *et al.*, 1971; Witkin and George, 1973) and sedimentation studies (Kanner and Hanawalt, 1970; Paterson *et al.*, 1971; Youngs *et al.*, 1974) indicate that DNA polymerase I is involved in the excision–repair pathway. Cooper and Hanawalt (1972a, b) demonstrated that, during the DNA resynthesis step of excision repair, newly synthesized patches of two distinct sizes are produced. The formation of large patches appeared to depend on the *recA* and/or *recB* genes, while DNA polymerase I was postulated to be involved in the production of shorter patches of repair-replicated DNA.

The existence of different pathways in excision repair has also been demonstrated by Youngs *et al.* (1974) who found that DNA polymerase I acts in a different branch of excision repair than the *recA*, *recB* or *exrA* gene products, as shown by viability and sedimentation experiments. Van der Schueren and Smith (1974) have found that 2,4-dinitrophenol inhibits a branch of excision repair that is dependent on the *exrA* gene but not on the *polA* gene, supporting the hypothesis that the *polA* and *exrA* gene products act on different branches of the excision–repair process.

In addition, the genetic studies of Nishioka and Doudney (1969, 1970) strongly suggest that the excision–repair process has two branches; one which is inaccurate and contributes to the yield of UV induced mutations, and a second, accurate branch which does not. The inaccurate branch of excision repair is apparently dependent on the *recA* (Miura and Tomizawa, 1968) *exrA* (Witkin, 1967) and probably *recB* and *recC* (Witkin, 1969a) gene products, since strains containing these mutations show little or no UV mutability.

The inhibitor present in agar–agar No. 3 did not seem to act on the DNA-polymerase-I-dependent pathway of excision repair, since an inhibitory effect comparable to that seen with wild–type cells was observed with UV irradiated *polA* 1 cells, which are deficient in DNA polymerase I. However, since no inhibitory effect was found when *recA*, *recB* or *exrA* cells were plated on agar–agar No. 3, it appears that some component of agar–agar No. 3 inhibits the *rec* and *exrA* gene-dependent branch of excision repair, which is present in wild–type and *polA* 1 cells.

If UV irradiated *E. coli* K–12 wild–type or *polA* cells were incubated in liquid minimal medium before plating, they rapidly became resistant to the inhibitory action of agar–agar No. 3 (Fig. 1). This rapid increase in the number of surviving cells cannot be due to rapid growth and/or cell division since no such increase in survivors was observed when the cells were assayed on washed Noble agar plates.

The time of incubation in liquid medium required before UV irradiated cells become resistant to the inhibitory effects of agar–agar No. 3 (i.e., 60–90 min, Fig. 1) must represent the time required to complete the repair that is subject to inhibition by agar–agar No. 3. Since this process appears to be excision repair, it is pertinent that the repair of the UV induced incision breaks in DNA (Kanner and Hanawalt, 1970) and completion of the excision of thymine dimers (Setlow and Carrier, 1964) require about the same length of time.

The *recA*, *recB* (Kapp and Smith, 1970; Youngs and Smith, in preparation) and *exrA* (Sedgwick and Bridges, 1972; Youngs and Smith, 1973a) genes also control the slow, growth-medium-dependent, Type III repair (Town *et al.*, 1973) of X-ray-induced DNA single-strand breaks. However, the unwashed agars have little or no inhibitory effect on the survival of X-irradiated cells. This suggests that components of unwashed agars inhibit some step in the *rec* and *exr* gene-controlled branch of excision repair of UV induced damage that is not essential to the *rec* and *exr* gene-controlled repair of X-ray-induced damage.

The large variation in survival of UV irradiated *E. coli* K–12 wild–type cells plated on medium solidified with different agars (Table 2) is a factor which must be considered in the design of any experiment testing the effects of repair inhibitors. For such experiments the use of agars containing components that inhibit repair should be avoided. In addition, it should be cautioned that agar sterilized at alkaline pH in phosphate buffer is rendered toxic to UV irradiated cells (Alcantara Gomes *et al.*, 1970).

Acknowledgements—We are grateful to John Miller for his excellent technical assistance. This work was supported by U.S. Public Health Service research grant CA-02896 and

research program project grant CA-10372 from the National Cancer Institute. One of us (E.V.D.S.) is a fellow of N.F.W.O. (Belgium).

ADDENDUM

Results similar to those described here for wild-type *E. coli* K-12 were also obtained for *E. coli* B/r when plated on Ionagar vs Noble agar plates.

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