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Liquid holding recovery and photoreactivation of UV-induced damage in *Streptococcus lactis*

Martine Lautier, Bouachanh Thammavongs, Yanick Auffray and Philippe Boutibonnes

Laboratoire de Génétique Microbienne, Equipe de Toxicologie Génétique, Institut de Recherche de Biologie Appliquée (I.R.B.A.), Université de Caen, Caen, France

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SUMMARY

Repair of ultraviolet-light-induced DNA damage in *Streptococcus lactis* has been examined. The wild-type strain and its derivative Lac$^-$ possess a dark repair system (maximal increase in survival of 4-fold). Enzymatic photoreactivation exists in the two strains but a weaker photoreactivability was found in the Lac$^-$ derivative (4 and 2-fold, respectively). Concomitant reduction of UV-induced mutagenesis (Rif^r marker) was also studied during these two repair phenomena. The absence of dark repair after saturation of photoreactivation suggests that photoreactivation is much more efficient with pyrimidine dimers as substrate.

INTRODUCTION

Microorganisms that are exposed to deleterious agents such as ultraviolet (UV, 254 nm) radiation may possess various repair mechanisms capable of restoring DNA integrity [3,21]. In *Escherichia coli,* UV-induced DNA damage is subject to repair by several enzymatic processes which can reverse the lethal and mutagenic effects of these photoproducts (principally cyclobutane-type dipyrimidine) [6].

Correspondence: P. Boutibonnes, Laboratoire de Génétique Microbienne, Equipe de Toxicologie Génétique, I.R.B.A., Université de Caen, 14032 Caen Cedex, France.

Most of these dimers can be repaired efficiently by error-free repair systems such as excision repair and enzymatic photoreactivation [5].

Liquid holding recovery (LHR) of irradiated cells is excision repair-dependent [17]. This dark repair phenomenon is characterized by a progressive enhancement of survival when the irradiated cells are held in buffer and plated at intervals on nutrient agar [7,11]; the increase in survival is accompanied by a decrease in mutagenesis.

Enzymatic photoreactivation (PR) occurs when UV-irradiated cells are exposed to visible light (>300 nm); as with LHR, an increase in survival with a concomitant decrease in mutagenesis is ob-

tained. The excision repair mechanism, initiated by a complex endonuclease, is distinguished by its broad substrate specificity [20]. PR is accomplished by a photolyase enzyme which monomerizes specifically the cyclobutane pyrimidine dimers when activated by visible light [4,12,14].

A number of bacteria appear to have these two repair systems, which are quite closely related to those of *E. coli* [19]; however, up to this time, no information has been available on the lactic acid bacteria, such as *Streptococcus lactis,* which play a vital role in the food industry (for a review see Ref. 10).

Such knowledge is essential for genetic studies as well as to provide a valuable foundation for developing effective mutagenesis procedures in these organisms.

Recently, we investigated the survival and mutagenesis of *S. lactis* cells after exposure to far-UV radiation (254 nm) and after alkylating agents [9].

The objective of the present study was to characterize DNA repair mechanisms operative in *S. lactis* exposed to UV light.

MATERIALS AND METHODS

Bacterial strains

S. lactis strain A45 was obtained from the CNRZ-INA collection (Centre National des Recherches Zootechniques, Institut National de la Recherche Agronomique, 78350, Jouy en Josas, France). A spontaneous Lac^- derivative of the wild type A45.00, cured of one plasmid, was isolated and called A45.02. The loss of the 37 MDa plasmid confers to A45.02 different UV-survival and mutagenesis properties [9].

Media and buffer

The strains were grown in M17 medium [18] containing: peptone (0.5%), biosoyase (0.5%), yeast extract (0.25%), beef extract (0.5%), ascorbic acid (0.05%), β-sodium glycerophosphate (1.9%) and $Mg\,SO_4 \cdot 7H_2O$ (0.025%). M17 agar medium (1.5% agar) was used to determine survival; rifampicin (obtained from Sigma) resistant mutations (Rif^r) were determined in the same medium supplemented by the addition of the antibiotic at 70 μg/ml. This forward mutation often arises via base substitution and involves a chromosomal gene [2]. The holding buffer used was 10 mM $MgSO_4$.

Irradiation conditions

For UV irradiation, exponentially growing cultures ($OD_{600\ nm}$ = 0.5; 5 × 10^8 cells/ml) were harvested by centrifugation (5000 × *g* for 10 min) and suspended in the holding buffer. Seven milliliters of the suspension were irradiated in a 90 mm petri dish at room temperature in the dark with constant stirring using a 6 W germicidal lamp (254 nm). UV doses were measured using a Black Ray UV meter, model J.225 (Ultraviolet Products, Inc.). All manipulations were carried out under a sodium lamp to avoid adventitious PR.

LHR

For LHR experiments, irradiated cells were held in buffer at room temperature in the dark and plated at intervals on nutrient agar for survival and Rif^r mutagenesis. A sample of UV-irradiated cells was maintained in ice in the dark as control.

PR

For PR experiments, UV-treated cells were immediately put in test tubes submerged in a beaker of ice and then were exposed to a 1000 W fan-cooled photoflood lamp at 100 cm distance for various times; a sample of UV-irradiated cells was maintained in ice in the dark as control.

Survival and Rif^r mutagenesis after LHR or PR

At various times during LHR or PR, samples were assayed for colony-forming units in M17 agar medium. To determine Rif^r mutation among holding buffer cells, treated cells were plated with M17 agar medium containing rifampicin. The plates for determining survival and Rif^r mutagenesis were scored after 48 h at 30°C and the mutation frequency was determined as the ratio of the number of mutants to the number of survivors.

RESULTS

S. lactis A45.00 and A45.02 were recently studied [9]: the derivative A45.02 appeared to be more resistant to UV light and less mutable than the parental strain. For LHR and PR experiments, a maximum recovery was obtained for A45.00 and A45.02, with 1% survival which corresponds to a UV fluence of 50 and 60 J/m^2, respectively.

Effect of LHR on UV survival and UV mutagenesis

Fig. 1 shows significant enhancement of the survival of UV-irradiated cells maintained at room temperature as compared to the samples kept in ice; the two strains showed LHR and the level of recovery, after 3 h, was found to be increased 3–4-fold; LHR reached its survival maximum 2 h after irradiation.

A slight decrease in mutagenesis (about 20–25%) was observed for the two strains, during the first hour of holding time; longer exposure did not amplify this phenomenon.

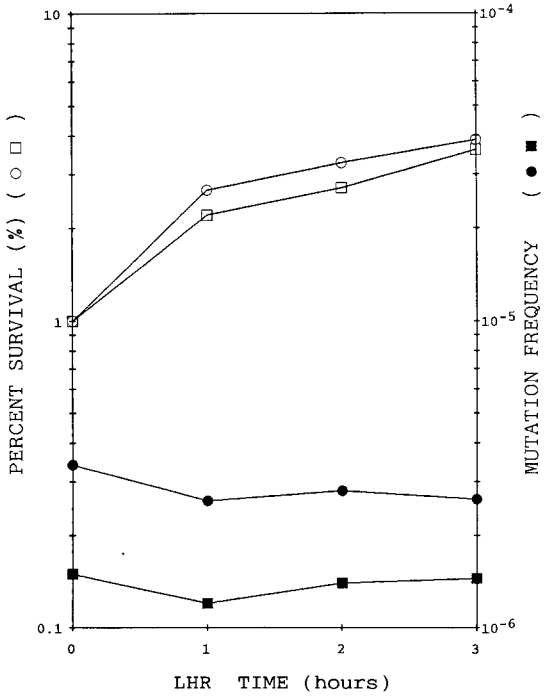

Fig. 1. Effect of LHR on survival (○,□) and mutagenesis to Rif^r (●,■) of *S. lactis* A45.00 (○,●) and A45.02 (□,■). Cells were irradiated with 254 nm UV-light fluences causing 1% survival. Mutation frequency values were based on the viability count (see Materials and Methods). Each point represents the average of at least four independent experiments.

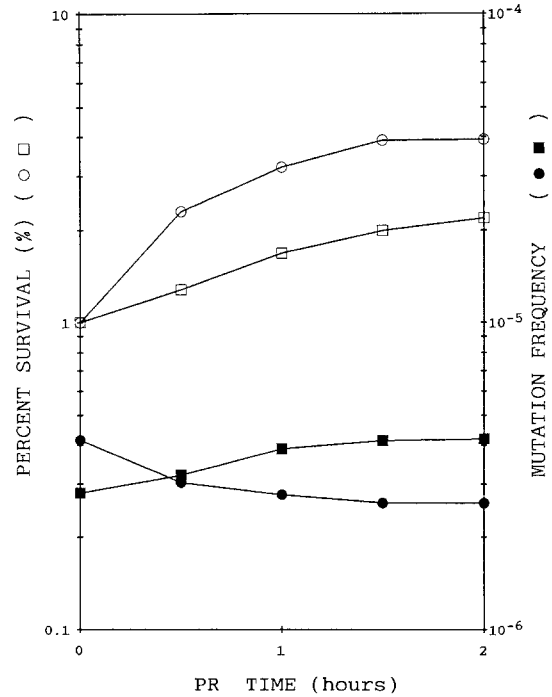

Fig. 2. Effect of PR on survival (○,□) and mutagenesis to Rif^r (●,■) of *S. lactis* A45.00 (○,●) and A45.02 (□,■). Cells were irradiated with 254 nm UV-light fluences causing 1% survival and then exposed to various time periods of photoreactivating light. Mutation frequency values were based on the viability count (see Materials and Methods). Each point represents the average of five independent experiments.

Effect of PR on UV survival and UV mutagenesis

As the time of exposure to PR light increased, there was a strong enhancement of the survival of UV-irradiated wild-type A45.00 cells (Fig. 2) as compared to the samples kept in the dark in ice (data not shown). PR reached its maximum 1.5 h after irradiation and the increase of survival was about 4-fold; a decrease in mutagenesis (about 30%) occurred during the first hour of PR (Fig. 2). The mutagenesis of A45.02 strain, however, increased by about 30% during the same period of PR and only a 2-fold enhancement of survival was obtained.

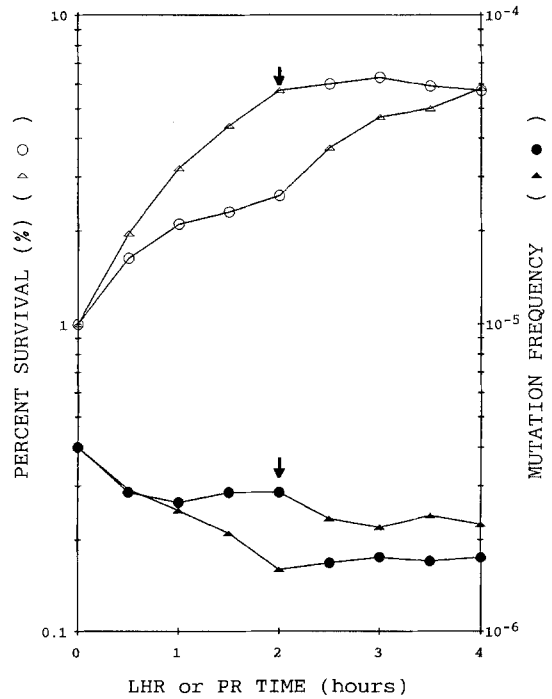

Fig. 3. Effect of LHR versus PR. UV-irradiated cells of *S. lactis* A45.00 were held in the dark as described in Fig. 1 (○,●) or illuminated with visible light as described in Fig. 2 (△,▲). Mutation frequency values were based on the viability count (see Materials and Methods). Survival is represented by the open symbols and mutagenesis to Rif^r by the closed symbols. The black arrow indicates the change of experimental conditions. Each point is the average of two independent experiments.

Comparison of LHR and PR capabilities in S. lactis A45.00

To examine whether, after maximum dark recovery, the irradiated cells could be further photoreactivated, a sample of UV-irradiated cells after 2 h of LHR was illuminated with visible light for varying periods and the number of colony-forming units was assayed. Dark recovery after saturation of PR was also examined.

Starting from the same initial survival of UV-irradiated cells, we observed a 'one-step curve' in survival and mutagenesis when the cells were subjected to PR before LHR (Fig. 3); in contrast, when LHR occurred before PR we noted a 'double-step curve'. However, the total amount of reactivation was independent of the order of the two repair mechanisms.

The PR observed after saturation of dark repair indicates that most of the lesions in DNA remain intact even after maximum dark recovery and can serve as a substrate for the photolyase. At the same time, the absence of LHR after maximum PR (Fig. 3) suggests that PR is much more efficient than LHR in reversing UV damage.

DISCUSSION

The results presented in this report show that the commercially important bacterium *S. lactis* is proficient in reversing UV-induced DNA damage by two error-free DNA repair mechanisms, PR and dark repair (LHR).

LHR in UV-irradiated cells occurs in the wild-type strain and in a Lac$^-$ variant of *S. lactis*. In this species, caffeine (2000 μg/ml) and acriflavine (5 μg/ml) do not prevent this phenomenon (data not shown), in contrast to what has been observed in *E. coli* B or in *recA E. coli* K12 [16].

However, these two cell lines are different in photoreactivating capability: in the wild type, more of the lethal effects than DNA lesions leading to Rif^r mutation are photoreactivable; in the Lac$^-$ derivative, this activity reverses to a lesser extent the lethal effects of photoproducts but not their mutagenic consequences.

It could be hypothesized that:

(i) the A45.02 derivative contains few photolyase molecules per cell as compared to the wild-type strain, and so prolonged exposure (up to 2 h) to photoreactivating light is required to attain full reactivation and a subsequent decrease in mutagenesis.

(ii) non-photoreactivable photoadducts represent the major lesion that induces SOS functions (UV-mutagenesis) [22] in A45.02.

In contrast, the absence of LHR observed after saturation of PR indicates that PR is much more efficient than dark repair. Assuming that the *S. lactis* photolyase specificity is the same as that of the *E. coli* enzyme, we suggest that the main type of photoproduct in UV-irradiated *S. lactis* is the *cis-syn* cyclobutyl pyrimidine dimer [3].

We have shown previously [9] that the *S. lactis* wild-type strain was more mutable after UV-irradiation than its Lac$^-$ derivative which has lost a 37 MDa plasmid. LHR therefore is equally effective in repairing lethal damage and damage leading to Rif^r mutations in these two strains. Both observations suggest that presumably an error-prone mechanism is triggered more effectively (or more quickly) in the wild type.

Correlations have been made between genetic transformability, non-photoreactivability and non-UV-mutability in some bacterial species [1,8] and especially in *S. pneumoniae* [15]. Since transformation has been recently demonstrated in *S. lactis* [13], the basis of these correlations is unclear in this species, which is photoreactivable (this work) and UV-mutable [9].

Therefore, we would like to point out that this is the first report which clearly shows the existence of error-free repair pathways (LHR and PR) in UV-irradiated *S. lactis*.

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