Repair of U.V. damages in Bacillus subtilis cultures competent for transformation: difference between competent and non-competent fractions

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ABSTRACT

The repair of U.V. damages to DNA in B. subtilis cultures competent for genetic transformation has been studied. The comparison of survival curves for competent and non competent fractions shows that: i) excision repair is more effective in competent than in non competent bacteria; ii) recombination repair is more effective in non competent than in competent bacteria. These facts support the hypothesis that metabolic conditions and, very likely, DNA replication play a role in the regulation of the efficiency of the two different mechanisms of repair.

INTRODUCTION

Reduced metabolism improves the efficiency of repair of U.V. damages in bacteria. As shown by Radman and Errera this fact can be related to a better efficiency of excision repair due to partial or complete inhibition of DNA synthesis.

On the contrary, it is well known that DNA replication is a prerequisite for recombination repair. Moreover it has been suggested that excision and recombination repair play a complementary role in the repair of U.V. damages to phage λ DNA, in that the efficiency of excision repair is related to temporary inhibition of λ DNA replication caused by U.V. damages.

As it is well known the metabolism of B. subtilis cells competent for genetic transformation, and in particular DNA synthesis, is very much reduced as compared with non competent ones. This property makes the two fractions (competent and non competent) of competent cultures of B. subtilis very suitable to compare, in this organism, the efficiency of repair mechanisms under different states of DNA metabolism.

The above considerations prompted us to study the U.V. sensitivity of competent and non competent fractions of B. subtilis strains both wild type or defective in either recombina-
ion or excision repair.

MATERIALS AND METHODS

Strains - The three isogenic strains of *B. subtilis* 168 M, GSY1026 (trpC2 metB4), GSY1025 (trpC2 metB4 recA1) and GSY1027 (trpC2 metB4 uvr-1) have been described by Hoch and Anagnostopoulos13.

*B. subtilis* 168 M trp+, of M.S. Fox's collection, was used to prepare donor DNA.

Media and Competent Cultures

Penassay Broth (Difco Antibiotic Medium No.3) was used for growing overnight cultures.

The minimal salts medium LS and the growth media 1 and 2 (called in what follows TM1 and TM2 respectively) described by Cahn and Fox14 were used to prepare competent cultures. TM1 and TM2 media were supplemented with 20 μg/ml of methionine.

Transformants were scored for the *trp* + marker in LS medium supplemented with 20 μg/ml of methionine, 0.02% Vitamin Free Casamino Acids (Difco), 0.5% glucose and 1.2% Bacto Agar (Difco). LB agar (1% Bacto Tryptone (Difco), 0.5% Bacto Yeast Extract (Difco), 1% NaCl and 1.2% Bacto Agar, pH 7.0) was used to determine the titer of viable bacteria.

Competent cultures were prepared from overnight cultures grown at 30°C for about twenty hours under good aeration. Cells of the overnight cultures were collected by centrifugation, resuspended at a concentration of about 2x10^7 bacteria/ml into TM1 and shaken at 37°C until the end of the exponential phase of growth (about 330 Klett units). This culture was diluted fivefold into TM2 and incubated with moderate shaking at 30°C for about 135 min., this being the approximate time of maximal competence under our conditions. For the transformation assays, MgSO_4 was added to a sample of the competent culture to a final concentration of 0.02 M. This sample was then mixed with about 2 μg/ml of donor DNA and incubated at 30°C for 30 min. with moderate shaking. DNA exposure was terminated by dilution into LS.

Fractionation of Competent Cells

The method of separating competent from non competent cells on Urografin (Schering, Milano) gradients is based on the
methods described by Cahn and Fox\textsuperscript{14}, Hadden and Nester\textsuperscript{15} and Singh and Pitale\textsuperscript{16}. A volume of 9 ml of a competent culture was layered on a 9-ml linear gradient of Urografin with refractive index ($n_{25}$) ranging from 1.3680 to 1.3720 over 1 ml of 50% Urografin cushion. Centrifugation was carried out in a Spinco SW27 rotor at 20°C for 15 min. at 24,000 rev/min. After centrifugation, the top and the bottom fractions of cells were removed with Pasteur pipette, collected on membrane filters (Millipore Corp., HA, 0.45 μm pore size) and washed thoroughly first with LS medium, then with 0.02 M MgCl$_2$ and finally with LS. Cells were then resuspended into TM2 and tested for competence.

**DNA Preparation**

\textit{B. subtilis} 168 M trp$^+$ was grown up to $3\times10^8$ cells/ml at 37°C in LS medium supplemented with 0.02% Vitamin Free Casamino Acids, 20 μg/ml tryptophan, 20 μg/ml methionine, 0.5% glucose. Bacteria were then harvested, washed three times with cold Tris EDTA buffer (0.1 M Tris (Sigma), 0.01 M Na$_2$EDTA (Sigma), pH 8.0) and resuspended into the same buffer with 0.3 M sucrose and 1 mg/ml lysozyme (Egg White, 3X, Sigma). This mixture was incubated at 37°C for 10 min. and lysed with 0.3% Sarcosyl (Geigy Industrial Chemicals, Ardley, N.Y.). The lysate was then incubated at 40°C with 1 mg/ml pronase (\textit{S. griseus}, SERVA, Heidelberg) for about 10 hours. DNA purification was carried out by isopycnic separation in CsCl (Merck, Suprapur) gradient. Fractions containing DNA were pooled and dialyzed against SSC (0.15 M NaCl, 0.015 Sodium Citrate).

**U.V. Irradiation**

For U.V. irradiation, bacteria were resuspended, after washing, in LS at a concentration of about $10^6$/ml, suitable to avoid screening during U.V. irradiation. More concentrated samples were microscopically examined at this stage. Bacterial clumps or long chains were rare.

Irradiation was performed using a 6W U.V. germicidal lamp (Philips TUV 57416 E/40MS). Irradiation rates, measured with a Latarjet dosimeter\textsuperscript{17} were 0.5 ergs/mm$^2$·sec. for bacterial strains GSY 1025 and GSY 1027 and 1 erg/mm$^2$·sec. for bacterial strain GSY 1026. Bacteria were irradiated in glass Petri dishes with gentle shaking.
RESULTS AND DISCUSSION

We shall often call here T (top) cells the cells of the competent fraction and B (bottom) cells the cells of the non-competent fraction.

The transformability both of cultures before fractionation, and of T and B cells have been tested in each experiment. The efficiency of separation was more than satisfactory, as shown in Table 1.

Table 1
Transformation frequency for \( \text{trp}^+ \) marker of competent cultures before fractionation and for T and B bands after fractionation.

<table>
<thead>
<tr>
<th>Bact. Strains</th>
<th>Before Fractionation</th>
<th>T Band</th>
<th>B Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSY 1026</td>
<td>(0.22-0.5) ( \times 10^{-2} )</td>
<td>(2.8-8.9) ( \times 10^{-2} )</td>
<td>5 ( \times 10^{-6} )</td>
</tr>
<tr>
<td>GSY 1025 recAl</td>
<td>(0.01-0.07) ( \times 10^{-2} )</td>
<td>(0.17-0.25) ( \times 10^{-2} )</td>
<td>1.5 ( \times 10^{-5} )</td>
</tr>
<tr>
<td>GSY 1027 uvr-1</td>
<td>(0.17-0.52) ( \times 10^{-2} )</td>
<td>(2.5-8.5) ( \times 10^{-2} )</td>
<td>1.3 ( \times 10^{-5} )</td>
</tr>
</tbody>
</table>

The results of our experiments on the relationship between the competent state and U.V. resistance are reported in Figg. 1, 2 and 3 for uvr rec, uvr rec, and uvr rec systems respectively. To facilitate the following discussion, we want to note here that, on the basis of the findings of Yoshikawa et al. 18 it is reasonable to expect that B cells, being metabolically active, have more chromosomal copies than T cells. This feature could result in a better rec+ mediated repair for non-competent cells, since it could allow them to eliminate U.V. damages by recombination events between non-replicated DNA regions 19 and/or by utilization of multiple postreplicative structures needed for recombination repair6.

i) GSY 1025 uvr+ rec-

Fig.1 shows that, when only excision repair is effective, B cells are more sensitive to U.V. irradiation, in spite of the fact that they have more chromosome copies than T cells. Since, as mentioned above, T cells are metabolically inert, this result suggests that the efficiency of excision repair depends on
the metabolic conditions and in particular on DNA replication for *B. subtilis*, as is observed for *E. coli* and for bacteriophage λ. 
Fig. 3 - Survival curves for the two fractions of competent cultures of B. subtilis GSY 1026 _uvr^+rec^+_.

Fig. 4 - Survival curves of T fractions of competent cultures of B. subtilis GSY 1025, GSY 1026 and GSY 1027.

ii) GSY 1027 _uvr^-rec^+

Delay of DNA synthesis should not, in general, cause any difference in U.V. survival when only recombination repair is effective. On the contrary curves reported in Fig. 2 indicate
that T cells have a U.V. sensitivity higher than B cells. Since this system is rec\(^{+}\), this result could be explained on the basis of the fact that non competent cells have more chromosomal copies than competent ones. Moreover, the present observation may parallel those reported by Radman \textit{et al.}\footnote{Radman and Errera (1973)} for phage \(\lambda\) single infection and by Radman and Errera\footnote{Radman and Errera (1973)} for \textit{E. coli}, i.e.: a decrease in survival if DNA synthesis was temporarily inhibited in excision deficient systems.

iii) GSY 1026 \textit{uvr}^{+}\textit{rec}^{+}

Curves reported in Fig.3 for wild type bacteria, in full agreement with those previously obtained by Cahn\footnote{Cahn (1973)} show a higher survival at low U.V. dose for the non competent fraction of the culture. Consistent with the results reported in Fig.1,2 one could then deduce that the advantage provided by recombination repair in non competent bacteria overtakes the advantage provided by excision repair in competent cells, due to the reduced rate of DNA synthesis.

The difference in the roles played by the two repair mechanisms in the two fractions of competent cultures of \textit{B. subtilis} is even more evident from curves reported in Fig.4 and 5, where the survival curves for T and B cells of all three sys-
tems are displayed together. It seems in fact very indicative that, over the range of irradiation examined, for T bands, \textsc{uvr}^{-}\text{rec}^{+} bacteria are the most sensitive, while, for B bands, \textsc{uvr}^{+}\text{rec}^{-} cells are the most sensitive.

We want to note here that our results are not easily comparable with the results reported by Mahler\textsuperscript{20} and Okubo and Romig\textsuperscript{21} who tested the U.V. sensitivity of competent cells by as-saying the number of transformants, having irradiated the bacteria after exposure to DNA.

In conclusion, we think that the reported results provide a further evidence of the interaction between DNA replication and repair of U.V. damages to DNA.

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