Recombination-Deficient \textit{Streptococcus sanguis}

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A UV-sensitive derivative was obtained from \textit{Streptococcus sanguis} Challis. The organism could be transformed with a number of small streptococcal plasmids at frequencies equal to, or 1 logarithm below, the transformation frequencies for the parent organism. However, transformation with chromosomal DNA was greatly impaired in the UV-sensitive derivative.

\textit{Streptococcus sanguis} Challis is a transformable organism useful for the study of streptococcal gene expression. Before the initiation of a series of gene-cloning experiments, it seemed desirable to obtain a recombination-deficient derivative to minimize the occurrence of classical host recombination events (1). A series of recombination-deficient mutants of \textit{S. sanguis} Challis has been described by Raina and Macrina (5). These derivatives were obtained after screening for sensitivity to methyl methanesulfonate (MMS), which occurred in 4\% of 7,000 mutagenized colonies, followed by screening for UV sensitivity (five colonies) and further selection for inability to be transformed with chromosomal DNA (one colony, called cipA9). However, in both cipA9 and its cipA-11 derivative obtained by those workers, transformation was severely impaired when both chromosomal DNA and DNA obtained from plasmids in the 5- to 7-megadalton range was used. This last impairment would reduce the usefulness of the derivatives in gene-cloning experiments.

We report here the isolation of an \textit{S. sanguis} derivative which appears to be recombination deficient by standard criteria (1, 7) but is capable of transformation with the streptococcal plasmids pVA1 and pVA736 (7.3 and 5.0 megadaltons, respectively), as well as with a \textit{Streptococcus-Escherichia coli} shuttle vector, pVA856 (9.2 megadaltons). The existence of such a strain suggests that the missing recombination function in our derivative affects chromosomal but not plasmid-mediated recombination events. The plasmids were provided by F. Macrina, Virginia Commonwealth University, Richmond (3, 5).

\textit{S. sanguis} 685 was mutagenized with 100-\mu g/ml \textit{N}-methyl-\textit{N}'-nitro-\textit{N}'-nitrosoguanidine as described by Yagi and Clewell (7), and the survivors were washed and suspended in Todd-Hewitt broth containing 2\% glucose and 0.1\% cysteine. \textit{S. sanguis} 685, which was provided by F. Macrina, is a derivative of the Challis strain V288 and contains the pVA380-1 helper plasmid (6). After replica plating onto the same medium (minus cysteine) containing 2\% agar, 24-h-old colonies were picked and replica plated onto two plates, one of which was irradiated for 40 s with UV light (160 ergs/cm² per s), and examined for viability at 48 h. Of 7,080 colonies tested, 1 colony, UV-48, was found to be UV sensitive by this criterion.

When DNA from a rifampin- or penicillin-resistant derivative of \textit{S. sanguis} 685 was used to transform the parent and UV-48, the transformation frequencies were 6 \times 10^{-2} and 8.9 \times 10^{-5}, respectively, for rifampin and 3.6 \times 10^{-4} and 2.16 \times 10^{-6}, respectively, for penicillin (Table 1). However, UV-48 could be transformed with pVA1, pVA736, and pVA856 (Table 1). The helper plasmid pVA380-1, which is present in the parent organism (6), was also present and apparently unaltered in UV-48, as determined by agarose gel electrophoresis (data not shown).

The UV sensitivities of the parent and of UV-48 are shown in Fig. 1A. Irradiation was performed with liquid cultures in petri dishes as described by Yagi and Clewell (7). Figure 1B shows the loss from [3H]thymidine-pretreated cells (100 \muCi/ml of medium) of 10\% of the trichloroacetic-acid-precipitable radioactivity after UV irradiation of the two strains. After irradiation for 160 s, a 5-ml sample of cells was diluted with 15 ml of warm medium and incubated at 37°C. At 180

<table>
<thead>
<tr>
<th>DNA source</th>
<th>Amt (\mu g/ml)</th>
<th>Transformation frequency</th>
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<tbody>
<tr>
<td>pVA1</td>
<td>4.3</td>
<td>1.39 \times 10^{-3}</td>
</tr>
<tr>
<td>pVA736</td>
<td>4.3</td>
<td>3.20 \times 10^{-2}</td>
</tr>
<tr>
<td>pVA856</td>
<td>1.0</td>
<td>1.04 \times 10^{-4}</td>
</tr>
<tr>
<td>Rif* S. sanguis</td>
<td>10.0</td>
<td>6.00 \times 10^{-2}</td>
</tr>
<tr>
<td>Pen* S. sanguis</td>
<td>10.0</td>
<td>3.60 \times 10^{-4}</td>
</tr>
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</table>

* Transforms from the plasmids were isolated on plates containing erythromycin (20 \mu g/ml). Transforms from rifampin resistance (Rif*)DNA were isolated on plates containing 0.1 \mu g of rifampin per ml. Transforms from penicillin resistance (Pen*)DNA were isolated on plates containing 0.03 \mu g of penicillin G per ml. The donor DNA was from a strain resistant to 0.55 \mu g of penicillin G per ml. The recipients had an MIC for penicillin G of 0.01 \mu g/ml. Transformation was performed as described by Lawson and Goode (2).

TABLE 2. Survival of 100 parental and 100 UV-48 colonies after exposure to MMS and UV irradiation

<table>
<thead>
<tr>
<th>Colony type</th>
<th>UV* MMS*</th>
<th>UV* MMS*</th>
<th>UV+ MMS+</th>
<th>UV+ MMS+</th>
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<tbody>
<tr>
<td>S. sanguis 685</td>
<td>88</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UV-48</td>
<td>7</td>
<td>0</td>
<td>65</td>
<td>28</td>
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</table>

* One hundred colonies from \textit{S. sanguis} 685 and its UV-48 derivative were replica plated and assayed for sensitivity to 1 mM MMS and to 60 s of UV irradiation. UV*: UV resistant.

* Corresponding author.
FIG. 1. (A) Effect of UV irradiation on viability of S. sanguis 685 (■) and its UV-48 derivative (▲). Cells in liquid culture were irradiated for the times indicated and then plated. (B) Effect of UV irradiation on DNA content of cells. Cultures (5 ml) of S. sanguis 685 (■) and of its UV-48 derivative (▲) were irradiated for 160 s, diluted to 20 ml, and examined for residual trichloroacetic acid-precipitable counts at various times. The points represent duplicate determinations.

FIG. 2. Effect of UV irradiation on survival of UV-48 and S. sanguis 685 (■). One hundred colonies were assayed. The symbols ▲, ■, and ▼ represent three successive passages of UV-48. Between passages a single UV-sensitive colony was grown overnight and plated, and 100 of these colonies were assayed.

of UV per cm² (60 s). These experiments were performed to compare our recombination-deficient derivative, obtained by a primary screen for UV sensitivity, with the recombination-deficient mutant of Raina and Macrina (5), which was obtained by a primary screen for MMS sensitivity. To our surprise, the results shown in Table 2 suggest that UV-48 is somewhat unstable with respect to MMS and UV sensitivity. It is not clear why the MMS phenotype is segregated as shown in Table 2. Raina and Macrina (5) observed segregation of MMS-sensitive (MMS') from MMS-resistant (MMS') clones. However, in their study the MMS' cells had been transformed with DNA from the MMS' cipA9 mutant, and the segregants were thought to be the outcome of additive integration. The occurrence of seven UV-resistant colonies may be due to cells surviving 60 s of UV irradiation on plates. In other experiments under identical conditions, UV-48 was killed most effectively after 120 s rather than after 60 s of irradiation (Fig. 2). In any event, the presence of a substantial number of MMS' clones in our UV-sensitive mutant provides further evidence that our recombination-deficient derivative differs from the cipA9 mutant reported by Raina and Macrina (5).

This work was supported by Public Health Service grant DE 05180 from the National Institute of Dental Research.

min, the UV-48 cells had solubilized 91.8% of their DNA, whereas the parental cells had solubilized only 12.4% of their DNA. Cells used in the experiment shown in Fig. 1A were used in the experiment shown in Fig. 1B.

We also examined 100 colonies of the parent and of the UV-48 strain for sensitivity to 1 mM MMS and to 9,600 ergs
We thank Alan Lee for technical assistance in the early phases of this work, Michael Pucci for helpful discussions, and Gregory Harvey for competent editorial work.

LITERATURE CITED