Isolation of a Recombination-Deficient Mutant of
Streptococcus lactis ML3†

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A recombinant-deficient mutant of Streptococcus lactis ML3 designated
MMS36 was isolated on the basis of its sensitivity to methyl methanesulfonate.
This mutant also displayed sensitivity to UV irradiation. The inability of MMS36
to mediate homologous recombination was demonstrated by transduction of
plasmid-linked lactose fermenting ability but not chromosomally mediated strep-
tomycin resistance.

Mutants deficient in host-mediated homolo-
gous recombination (Rec−) display characteris-
tic sensitivities to UV irradiation and mutagens,
such as N-methyl-N-nitro-N-nitrosoguanidine
and methyl methanesulfonate (MMS) (5, 6).
These phenotypic characteristics have recently
been exploited to isolate recombination-defi-
cient mutants of Micrococcus radiodurans (11),
Staphylococcus aureus (4, 5), and Streptococ-
cus faecalis (13). Such recombination-deficient
mutants have proved useful in determining the
extent to which host recombination is involved
in observed recombination events (1). To initiate
characterization of the recombination events
leading to the formation of a recombinant 60-
megadalton plasmid in transconjugants of Strep-
tococcus lactis ML3 (12), we isolated a recombi-
nation-deficient mutant of S. lactis ML3. The
methodology utilized to isolate and characterize
this strain is reported below.

Strains of S. lactis ML3 were maintained in
Elliker broth (3). Ten-milliliter cultures propa-
gated for 4 h at 32°C from a 2% inoculum of a 6-
h culture were used for mutagenesis, UV irradia-
tion, and transduction experiments. Mutation
was accomplished by exposing cells washed
with 0.85% saline to 1.0% ethyl methanesulfo-
nate in 30 mM K2HPO4, pH 8.0, for 60 min at
32°C. After mutation, cells were washed and
inoculated into Elliker broth for phenotypic
expression. These mutated cultures were then
plated out on Elliker agar for replica plating.

To screen for recombination-deficient mu-
tants, we replica plated the colonies onto fresh
Elliker agar plates containing MMS at a concen-
tration of 0.04 μl/ml. This concentration of MMS
reduced the growth rate of S. lactis ML3 and
should kill recombination-deficient mutants of
S. lactis ML3. A total of six MMS-sensitive
mutants were isolated from the screening of
approximately 15,000 colonies. The minimal in-
hibitory concentrations of MMS for these mu-
tants are presented in Table 1. It is clear that
strain MMS36 was the most sensitive to MMS.

The UV sensitivity of these strains was deter-
mined to confirm the other phenotypic charac-
teristic of recombination-deficient mutants. To
determine UV sensitivity, 1 ml of cells was
washed in 0.85% saline and resuspended in 10 ml
of saline. Ten milliliters of this cell suspension
containing 106 CFU/ml was transferred to a glass
petri dish (100 by 15 mm) and exposed to UV
irradiation during constant swaying. The UV
source was a 15-W General Electric germicidal
lamp located 38.5 cm above the cell suspension.
All the MMS-sensitive mutants were also UV
sensitive. The UV dose-survival curve for
MMS36 is presented in Fig. 1.

To determine whether these MMS-sensitive,
UV-sensitive mutants were recombination defi-
cient, we performed transduction experiments.
In a recombination-deficient strain, chromo-
mosomal markers introduced by transduction can-
not be integrated and expressed. However,
since chromosomal integration is not required
for expression of plasmid markers, these mark-
ers can be transduced into a recombination-
deficient strain. Goering used this approach to
characterize recombination-deficient mutants of
S. aureus (4). In transduction experiments with
MMS-sensitive mutants of S. lactis ML3, strep-
tomycin resistance was employed as a chromo-
osomal marker, and lactose metabolism was
employed as a plasmid-linked characteristic.
Bacteriophage were induced from appropriate

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Strains of *S. lactis* C2 by UV irradiation as described by McKay and Baldwin (7). Streptomycin resistance-transducing phage were induced from *S. lactis* LM0220 (2); lactose-transducing phage were induced from *S. lactis* transductants 8 and 55 (8). Transduction was performed as described by McKay et al. (9). Streptomycin-resistant transductants were plated on agar containing 5% nonfat dry milk and 1% glucose to permit phenotypic expression and then replica plated onto lactose-bromocresol purple indicator plates (10) containing 600 µg of streptomycin per ml and 0.5% lactose. Lactose-positive (*Lac*⁺) transductants were plated directly onto lactose-bromocresol purple indicator plates.

The frequency with which the MMS-sensitive strains were transduced to streptomycin resistance is presented in Table 1. Since control plates containing no phage gave a background of zero to two streptomycin-resistant colonies per plate, the data presented represent the number of transductants above this background. Strain MMS36 could not be transduced to streptomycin resistance. Additional transduction experiments utilizing concentrated phage preparations verified this observation: streptomycin resistance was transduced to *S. lactis* ML3 at a frequency of 4.68 × 10⁴ transductants per ml of phage, whereas no streptomycin-resistant transductants of MMS36 were observed.

To examine the frequency with which plasmid-linked lactose metabolism is transduced, lactose negative (*Lac*⁻) derivatives of *S. lactis* ML3 and MMS36 were employed. The absence of the 33-megadalton lactose plasmid from spontaneous *Lac*⁻ derivatives of *S. lactis* ML3 (LM3301) and MMS36 was verified by agarose gel electrophoresis (data not shown). The *Lac*⁻ derivatives of *S. lactis* ML3 and MMS36 were transduced to *Lac*⁺ at average frequencies of 1.79 × 10⁴ and 2.21 × 10⁵ transductants per ml of phage, respectively. This observation documents transduction of a plasmid-linked marker to MMS36. Although the transduction frequency of the *Lac*⁻ MMS36 strain was 10-fold lower.

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**TABLE 1. Characterization of *S. lactis* ML3 and its MMS-sensitive derivatives**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Minimal inhibitory concn (µ/ml) of MMS</th>
<th>No. of Str⁺ transductants per ml Expt 1</th>
<th>No. of Lac⁺ transductants per ml Expt 1</th>
<th>Expt 2a</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. lactis</em> ML3</td>
<td>0.20</td>
<td>1,256</td>
<td>46,800</td>
<td>14,800</td>
</tr>
<tr>
<td>MMS4</td>
<td>0.02</td>
<td>248</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMS22</td>
<td>0.02</td>
<td>233</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMS29</td>
<td>0.02</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMS30</td>
<td>0.02</td>
<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMS36</td>
<td>&lt;0.01</td>
<td>0</td>
<td>0</td>
<td>1,810</td>
</tr>
<tr>
<td>MMS37</td>
<td>0.02</td>
<td>353</td>
<td></td>
<td>2,610</td>
</tr>
</tbody>
</table>

a Transducing phage isolated by UV induction are defective and, consequently, cannot be titered conventionally (7). Therefore, transduction frequencies are reported as the number of transductants observed per milliliter of the specific lysate used. Streptomycin resistance is chromosomally mediated; lactose fermenting ability is plasmid mediated.

b The transducing phage was concentrated with polyethylene glycol (molecular weight, 20,000).

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**FIG. 1.** Dose-survival curves for *S. lactis* ML3 and MMS-sensitive mutant MMS36 after exposure to UV irradiation.
than that of the Lac− S. lactis ML3, these results are consistent with the transduction frequencies observed for plasmid-linked markers of Rec− and Rec+ strains of S. aureus (4).

In conclusion, a recombination-deficient mutant of S. lactis ML3, designated MMS36, was isolated by screening mutated colonies for sensitivity to MMS. Strain MMS36 displayed sensitivity to UV irradiation, which is a characteristic of many recombination-deficient mutants. Transduction of a plasmid-linked marker, but not a chromosomally linked marker, confirmed the recombination-deficient nature of this mutant. This Rec− mutant will play a vital role in characterizing the recombinational events occurring in S. lactis ML3, as well as other strains of dairy streptococci.

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LITERATURE CITED