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Recombination-Deficient Mutant of *Streptococcus faecalis*

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An ultraviolet radiation-sensitive derivative of *Streptococcus faecalis* strain JH2-2 was isolated and found to be deficient in recombination, using a plasmid-plasmid recombination system. The strain was sensitive to chemical agents which interact with deoxyribonucleic acid and also underwent deoxyribonucleic acid degradation after ultraviolet irradiation. Thus, the mutant has properties similar to those of *recA* strains of *Escherichia coli*.

The absence of known transducing phages and the inability to transform has severely limited genetic studies in *Streptococcus faecalis*. Such studies are now possible, however, due to the relatively recent discovery of conjugative plasmids in this species (5, 8, 11, 12, 16, 20). These plasmids are responsible for such traits as drug resistance and the production of hemolysin and bacteriocin activities. Some confer mating responses to specific sex pheromones produced by recipient strains (4, 6). In addition, certain conjugative plasmids can mobilize nonconjugative plasmids (5, 19) as well as chromosomal markers (7).

Genetic studies are frequently facilitated by the use of recombination-deficient mutants. Such variants are useful in determining the extent to which certain genetic events are dependent on classical host recombination machinery (1, 9). Whereas recombination-deficient variants of *Streptococcus pyogenes* (15) and pneumococci (21) have been reported, such mutants have not been reported for other members of the genus *Streptococcus*. We report the characterization of a derivative of *S. faecalis* which is defective in recombination. The strain was obtained on the basis of sensitivity to UV irradiation (9) and was shown to have a number of properties similar to Rec$^-$ derivatives of other species. A homologous plasmid-plasmid recombination system was used to detect the deficiency in recombination.

MATERIALS AND METHODS

Bacteria and media. *S. faecalis* strains used in this work are listed in Table 1. The primary strain in this study is the plasmid-free JH2-2, a derivative of the clinical isolate JH2 (12) having chromosomal mutations conferring resistance to rifampin and fusidic acid. All of the other strains are derivatives of JH2-2 with the exception of JH2SS, which is a derivative of JH2 having chromosomal mutations conferring resistance to streptomycin and spectinomycin. Plasmid-containing strains were constructed as previously described (5). With the exception of the mating experiments, the broth medium used was M9-YE (23). For the mating, N2GT (6) was used. For making agar plates Difco Penassay broth (antibiotic medium no. 3) was used.

Materials. The materials and their sources were as described previously (3, 5). Spectinomycin was a gift from The Upjohn Co. Mitomycin C was from Calbiochem, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was obtained from the Aldrich Chemical Co.

Isolation of UV-sensitive mutants. An overnight culture (0.25 ml) of strain JH2-2 was inoculated into 3.25 ml of M9-YE broth and incubated (37°C) to mid-log phase (80 Klett units, using a Klett-Summerson colorimeter with a no. 54 filter). The cells were then pelleted and resuspended in 2.5 ml of M9-YE broth containing 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml and incubated for 30 min at 37°C. The cells were washed thoroughly in broth, and appropriate dilutions were plated onto Penassay agar plates. After 24 h at 37°C, the plates were each replicated onto two plates, one of which was exposed to UV irradiation for 40 s (see below). Those cells which gave rise to colonies on the unirradiated plates but failed to grow on the irradiated plates were purified and checked again for UV sensitivity.

UV irradiation. Irradiation was carried out at a distance of 30 cm, using a General Electric G8T5 germicidal lamp. This was the case for the mutant selection and the UV sensitivity study, as well as the DNA degradation study. In the latter two cases, the liquid culture (5 ml) was in a 9-cm-diameter glass petri dish, and the cells were swirled gently during the UV exposure.

DNA degradation after UV irradiation. DNA degradation experiments were carried out by using a modification of the method of Inoue et al. (10). Cells (10 ml) were grown in the presence of [^{3}H]thymidine (5 μCi/ml) for several generations to mid-log phase (80 Klett units). They were then washed twice with broth and resuspended in 50 ml of broth containing 30 μg of unlabeled thymidine per ml. After incubation for 50 min, the cells were pelleted and concentrated fourfold in broth (containing 30 μg of unlabeled thymidine per ml). A 5-ml portion of this suspension was irradiated with UV for 40 s and another 5 ml served as an unirradiated control. After irradiation, the culture was added to 15 ml of broth (containing unlabeled thymidine, 30 μg/ml) and incubated at 37°C. Samples (1 ml)

TABLE 1. *S. faecalis strains used in this study*

Strain	Comments (reference)
JH2-2	Plasmid-free strain resistant to rifampin and fusidic acid (12)
JH2SS	Plasmid-free strain isogenic with JH2-2; resistant to streptomycin and spectinomycin
UV202	UV- and mitomycin C-sensitive mutant of JH2-2
MC-1	Revertant of UV202 resistant to UV and mitomycin C
YY1	Transconjugant of JH2SS harboring plasmid pAD1::Tn*917*(Em) (18)
YY2	Transconjugant of JH2-2 harboring plasmid pAD1::Tn*916*(Tc)
YY3	Transconjugant of UV202 harboring plasmid pAD1::Tn*916*(Tc)
YY4	Transconjugant of MC-1 harboring plasmid pAD1::Tn*916*(Tc)

were removed and mixed with 1 ml of cold (4°C) 10% trichloroacetic acid containing unlabeled thymidine (100 μg/ml). After 2 h on ice, the cells were collected on filters (Millipore Corp., type HA), washed with 50 ml of cold 10% trichloroacetic acid, and dried. The radioactivity remaining on the filters was measured with a scintillation counter (3).

Mating experiments (recombination). Before mating, 0.25 ml of freshly grown (mid-log phase) donor cells was diluted 10-fold into a 1:1 mixture of fresh N2GT broth and culture filtrate of a log-phase culture of strain OG1-10 (plasmid-free; as a source of the sex pheromone cAD1 [6]) and incubated for 20 min at 37°C. Matings with a fresh log-phase culture of "recipients" were then carried out for 2 h in N2GT broth (1 donor per 10 recipients). After mating, the cell mixtures were plated on appropriate selective media. The drug concentrations were: rifampin, 25 μg/ml; fusidic acid, 25 μg/ml; tetracycline, 5 μg/ml; and erythromycin, 50 μg/ml.

RESULTS

Mutagenized cultures of *S. faecalis* strain JH2-2 were replica-plated and exposed to UV irradiation to obtain UV-sensitive mutants. Two of 744 colonies examined were UV sensitive. One of these mutants, strain UV202, was selected for further characterization as described below.

UV202 was also more sensitive to mitomycin C than the parental strain. (The minimal inhibitory concentration on agar plates for mitomycin C was 0.25 μg/ml for the mutant strain, compared to 2.0 μg/ml for the parent strain.) It was possible to isolate a revertant, designated MC-1, by plating on a medium containing 1.0 μg of mitomycin C per ml. (Revertants appeared at a frequency of 10^{-8}.)

Strain UV202 was much more sensitive than the parental strain to UV irradiation (Fig. 1). The mitomycin C-resistant revertant (MC-1) regained its resistance to UV, thus indicating that a single genetic lesion is responsible for both

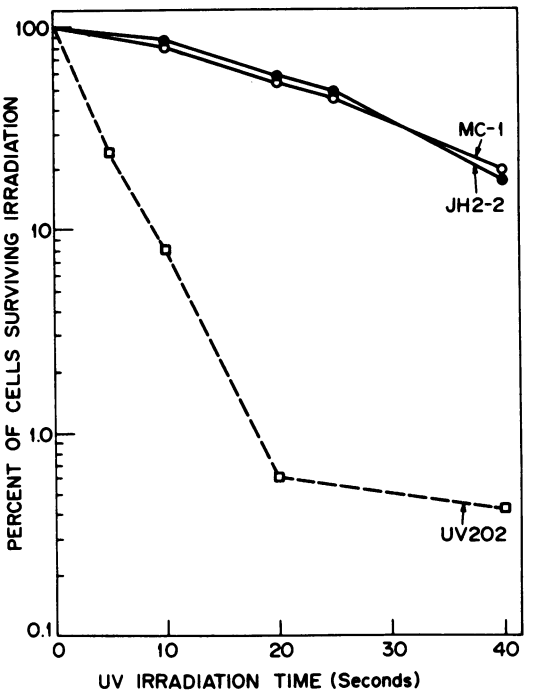

FIG. 1. *UV sensitivity of S. faecalis strains. The cells were irradiated as indicated in the text. Symbols: ●, parental strain JH2-2; □, mutant UV202; ○, revertant MC-1.*

properties. The mutant strain was also extremely sensitive to exposure to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (100 μg/ml), which after a 60-min exposure of a log-phase culture, resulted in an approximately 10^3-fold greater loss in viability compared with JH2-2. (MC-1 was resistant to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.)

Whereas strain JH2-2 has a doubling time of about 35 min (in M9-YE), UV202 grew slower, with a doubling time of 55 min. In log phase, the number of colony-forming units as a function of culture mass was similar for both parent and mutant; however, in the case of the mutant, a dramatic drop-off was observed after the cells entered stationary phase. It is also noteworthy that, unlike the parental strain, UV202 cells changed from a typical diplococcal appearance to chains of six to eight cocci after several hours in stationary phase.

Since certain recombination-deficient mutants in other species are known to undergo enhanced DNA breakdown upon exposure to UV, this trait was examined in the case of UV202. Cells labeled for several generations with [^{3}H]thymidine were irradiated, and loss of acid-precipitable DNA was measured (Fig. 2). The mutant strain showed significantly more degra-

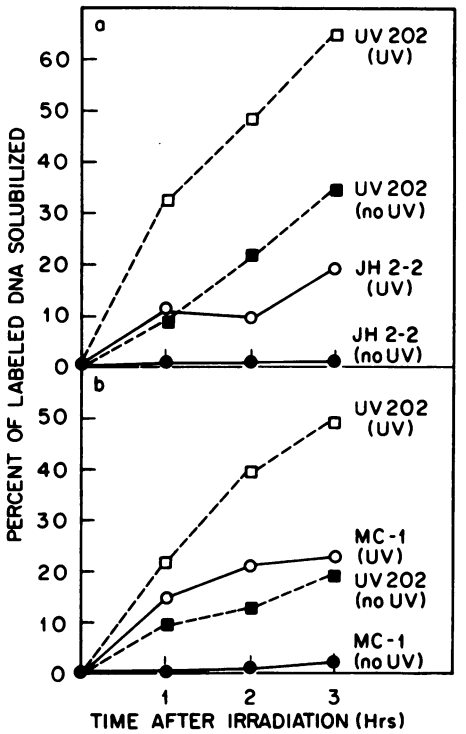

FIG. 2. *DNA degradation in strains JH2-2, UV202, and MC-1 after UV irradiation. Cells labeled with [^{3}H]thymidine were either UV irradiated (for 40 s), or untreated. During subsequent incubation, portions of cells were removed and suspended in cold trichloroacetic acid. Radioactivity remaining in cold acid-precipitable DNA on filter membranes was measured (a and b represent two separate experiments).*

dation than the parent or the revertant. In addition, the mutant strain underwent a significant degree of degradation (compared with the parent or revertant) even in the absence of irradiation.

Recombination of homologous plasmids. The plasmid pAD1 (molecular weight, 35×10^6) determines hemolysin and bacteriocin production and is conjugative (19). Derivatives of this plasmid containing the tetracycline (Tc) resistance transposon, Tn*916* (molecular weight, 9.5×10^6), and the erythromycin (Em) resistance transposon, Tn*917* (molecular weight, 3.3×10^6), have been constructed (18; A. E. Franke and D. B. Clewell, manuscript in preparation). One of each type was used for the plasmid-plasmid recombination experiments. The two plasmids are designated pAD1::Tn*916*(Tc) and pAD1::Tn*917*(Em). Since recombination between these two plasmids occurs readily, we assume that the two transposons are located at different sites on the plasmid.

Strain YY1, i.e., JH2SS [pAD1::Tn*917*(Em)], was used as a donor strain carrying the erythromycin-marked plasmid; it has the chromosomal mutations for resistance to streptomycin and spectinomycin. Matings were carried out in parallel with the recipient strains YY2, YY3, and YY4 (Table 2). When selection was for recipients which had received pAD1::Tn*917*(Em) but which were not forced to maintain the tetracycline resistance marker (i.e., where plasmid recombinants were not selected), the number of transconjugants per recipient cell (at the end of the mating time) was essentially the same for each of the three matings. Five such transconjugants were examined in each case and found to be sensitive to tetracycline, reflecting the incompatibility of the two plasmids. When plasmid recombinants were selected (i.e., by selecting for both erythromycin and tetracycline resistances), the UV202 host strain gave rise to approximately 100-fold fewer recombinants compared with the JH2-2 and MC-1 host strains. Erythromycin- and tetracycline-resistant recombinants of the JH2-2 recipients were shown by subsequent matings to have both markers linked; sedimentation analyses (data not shown) revealed that the recombinant plasmids were of a size of approximately 47 megadaltons, corresponding closely to the predicted size of a recombinant.

The possibility that recombination involves transposition of one of the transposons seems highly unlikely, since transposition generally occurs at frequencies of 10^{-5} to 10^{-7} between two coexisting replicons. The data of Table 2 show that, in the case of JH2-2 and MC-1, recombination occurs at a frequency of approximately 10% (i.e., 1 of 10 newly introduced plasmids recombines with, rather than displaces, the resident plasmid). Although it is conceivable that after the conjugal transfer of the donor plasmid transposition from the newly introduced DNA might be enhanced, we would have expected similar behavior in all three recipients. (Trans-

TABLE 2. *Recombination of pAD1::Tn916(Tc) and pAD1::Tn917(Em)*[a]

Recipient strain[b]	Frequency of transconjugants per donor selected on:	
	Em Rif Fus	Em Tc Rif Fus
YY2	4.4×10^{-6}	5.1×10^{-7}
YY3	2.0×10^{-6}	0.5×10^{-8}
YY4	5.6×10^{-6}	5.6×10^{-7}

[a] Broth mating (2 h) used strain YY1 as a donor.
[b] All recipient strains were chromosomally resistant to rifampin (Rif) and fusidic acid (Fus).

position generally occurs by *rec*-independent processes [13].)

DISCUSSION

The results presented here have concerned the derivation of a UV-sensitive variant (strain UV202) of *S. faecalis* strain JH2-2, which has properties very similar to those found for certain classes of *rec* mutants of other species. The ability to revert suggests that the original lesion was a point mutation. The mutant resembles *recA* strains of *Escherichia coli* (1), which, like similar mutants in *Haemophilus influenzae* (14), *Bacillus subtilis* (17), *Staphylococcus aureus* (10, 22), and *S. pyogenes* (15), are sensitive to UV irradiation and chemical agents which interact with DNA. Such strains also have been shown to undergo DNA degradation upon exposure to irradiation; UV202 also exhibited this trait.

Plasmid-plasmid recombination in UV202 was about 100-fold less frequent than in the case of the parent or the revertant, a reduction typical of Rec⁻ mutants of other species (1). Thus, we have succeeded in deriving a Rec⁻ strain of *S. faecalis* which is now available for various types of genetic studies in this species.

Strain UV202 has already been useful in analyzing the amplification of the small tetracycline resistance plasmid pAMα1 (2, 23). Previous studies had shown that the generation of tandem repeats of the resistance determinant during extended cellular growth in the presence of tetracycline is probably due to recombinational events facilitated by two small direct repeats (indicated RS1, for recombination sequence) flanking the resistance determinant (23, 24). We have now shown that amplification will not occur when pAMα1 is harbored by strain UV202 (25), indicating a dependence on host recombination machinery.

Genetic studies in *S. faecalis* are currently in their infancy; however, we anticipate a surge of activity in this area. We expect that in the future Rec⁻ strains such as UV202 will play an important role in such studies.

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