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Short Communication

The recombination deficient *Enterococcus faecalis* UV202 strain is a *recA* mutant

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Abstract

The *recA* gene of the recombination deficient *Enterococcus faecalis* strain UV202 was sequenced and found to encode a glycine to aspartic acid mutation at amino acid 265. Both the UV sensitive and recombination deficient phenotypes of the UV202 strain were complemented by expression of the wild-type *recA* gene cloned under the control of the nisin-inducible promoter of an expression vector.

Keywords: Recombination; DNA repair; *recA*; Amplification of plasmid-encoded antibiotic resistance genes

1. Introduction

The recombination deficient *Enterococcus faecalis* strain UV202 was isolated in 1980 (Yagi and Clewell, 1980b) and has been used ever since in enterococcal studies requiring limited recombination potential, such as complementation studies and studies requiring independent maintenance of multiple plasmids. The UV202 strain was also used to demonstrate the involvement of homologous recombination in the amplification of tetracycline resistance on the pAMα1 plasmid (Yagi and Clewell, 1980a). UV202 was isolated from the JH2-2 strain by chemical mutagenesis with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and shown to have increased sensitivity to UV light as well as recombination defects (Yagi and Clewell, 1980a,b). While it was assumed that UV202 was defective in some function of the recombination machinery, the precise mutation was never defined. The RecA protein is the central player in most recombination reactions and its gene is highly conserved in most bacterial cells, including *E. faecalis* (McGrew and Knight, 2003). To determine if the recombination defect in UV202 was due to a defect in RecA function, the *recA* gene was sequenced from both UV202 and JH2-2. The *recA* sequence from JH2-2 was found to be identical to the published sequence from strain V583 (Paulsen et al., 2003) while that from UV202 contained a single point mutation resulting in a glycine to aspartic acid substitution at amino acid 265. To determine whether this mutation was responsible for the recombination deficient phenotype, a promoter-less version of the JH2-2 *recA* gene was cloned

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downstream of the nisin-inducible promoter of pMSP3535 and shown to complement the UV sensitive phenotype and support antibiotic gene amplification after nisin induction.

2. Procedures

Enterococcus faecalis strains used were UV202 (Yagi and Clewell, 1980b) and JH2-2 (Jacob and Hobbs, 1974) and were cultured routinely in Todd Hewitt Broth (THB, Sigma) medium at 37 °C. *Escherichia coli* DH5α competent cells were purchased from Invitrogen, (Carlsbad, CA) and grown in Luria–Broth (LB, Ausubel et al., 1998) at 37 °C. For preparation of solid media, agar (Sigma) was added at 1.8%. Cloning vectors used were pGEMT-Easy (Promega) and pMSP3535 (Bryan et al., 2000). Ampicillin (100 μg ml^{-1}) was used for selection of pGEMT-Easy in *E. coli*. For selection of pMSP3535 erythromycin was used for selection at 150 μg ml^{-1} in *E. coli* and 10 μg ml^{-1} in *E. faecalis*.

Plasmid DNA was purified from *E. coli* using the Bio-Rad Quantum Prep plasmid mini-prep kit and from *E. faecalis* using a previously described alkaline lysis procedure (Weaver and Clewell, 1988). *E. faecalis* genomic DNA was purified as previously described (Pospiech and Neumann, 1995). Restriction enzymes and T4 DNA ligase were purchased from New England BioLabs and used according to manufacturer's directions. Gel purification of restriction fragments was performed using the QIAEX II Gel Extraction Kit (Qiagen) according to manufacturer's instructions. Plasmid DNA was introduced into Subcloning Efficiency DH5α chemically competent cells (Invitrogen) according to manufacturer's instructions and into *E. faecalis* by electroporation as previously described (Cruz-Rodz and Gilmore, 1990). Sequencing was performed by Lone Star Labs (Houston, TX). PCR was performed using the PCR Supermix kit (invitrogen) according to the manufacturer's instructions. Primers used were: 5′-*recA*, 5′-GGCAGATGATCGTAAAGTGG-3′; 3′ *recA*, 5′-CTATTCATCTAAGGGTAATTC-3′; *recA*-RBS, 5′-CG*GGATCC*AAGGAGGATATTCCATTGGC-3′; 5′-*rnc*, 5′-GGTTACCATGGACAATCAGTTAACAAC-3′; 3′-*rnc*, 5′-CCATTCGGACCAACCACTGC-3′.

For RNA purification and UV exposure, *E. faecalis* cultures were grown overnight in THB medium with the appropriate antibiotic, then diluted 1:10 in the morning in fresh medium and grown for 1 h at which point nisin (250 ng/ml) was added to induced cultures. Incubation was then continued for another hour. For RNA purification, cells were harvested and RNA was purified using the FastRNA Pro Blue Kit (Qbiogene) according to the manufacturer's directions. RNA (0.2 μg) was then treated with DNA-*free* (Ambion) to remove residual DNA in the sample. Semi-quantitative PCR was performed using the Qiagen OneStep RT-PCR Kit according to the manufacturer's instructions including the no-RT control. Densitometry was performed on ethidium bromide stained gels using a Typhoon Imager, model 9410 and analyzed using ImageQuant software. For UV exposure cells were serially diluted and either 100 μl aliquots were spread or 10 μl aliquots were spotted onto THB agar plates. Plates were then exposed to various UV light levels in a Spectrolinker XL-1000 UV crosslinker (Spectronics).

3. Identification of a recA mutation in UV202, and cloning and expression of the wild-type gene

The *recA* genes of the JH2-2 and UV202 strains were amplified from genomic DNA by polymerase chain reaction (PCR) using the 5′-*recA* and 3′-*recA* primers. Products were cloned into pGEMT-Easy and sequenced using both M-13 forward and reverse primers to determine the complete sequence. The JH2-2 *recA* sequence was found to be identical to the published sequence for *E. faecalis* strain V583. The UV202 *recA* sequence contained a single nucleotide change at residue 797 from a G to an A which results in a glycine-to-aspartic acid change at amino acid 265 (numbered without including the initiating Met according to convention). This glycine is well-conserved in *recA* sequences from other organisms. The analogous amino acid in *E. coli* is G268 which is located between Y264, implicated in ATP contact, and the C-terminal domain beginning at residue 270, implicated in gating dsDNA (McGrew and Knight, 2003). Although to our knowledge no mutation has been previously isolated in this residue in either *E. coli* or *Bacillus subtilis*, it seemed likely that this mutation could lead to inactivation of the RecA protein product.

To test whether this mutation was responsible for the phenotype of UV202, the wild-type *recA* gene was amplified from JH2-2 using the *recA*-RBS primer and the 3′-*recA* primer. The *recA*-RBS primer is complementary to sequences further upstream of the *recA* gene than the 5′-*recA* primer and includes the putative ribosome binding site as well as a *Bam*HI restriction site to be used for