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Plasmid 55 (2006) 158–163
PLASMID
www.elsevier.com/locate/yplas

Short Communication

Entry exclusion activity on conjugative plasmid pVT745

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Received 19 May 2005, revised 6 July 2005
Available online 13 September 2005
Communicated by Ellen L. Zechner

Abstract

Conjugative plasmid transfer into a recipient cell containing the same or a closely related plasmid is inhibited by a mechanism called entry or surface exclusion. The function of entry exclusion is to reduce unproductive conjugation. The current study assessed the exclusion activity on conjugal plasmid pVT745 by conducting mating experiments with genetically distinguishable derivatives of this plasmid. Our results demonstrate that a single gene, *magB05*, that is located in a gene cluster associated with mating pore formation, is responsible for the entry exclusion phenotype of pVT745.

Keywords: Conjugation; Entry exclusion; Plasmid; *Actinobacillus actinomycetemcomitans*

Bacterial conjugation involves the transfer of a plasmid from a donor to a recipient strain. The presence of a plasmid in the recipient that is either identical or closely related to the plasmid being transferred usually results in a significantly reduced rate of transmission to prevent futile conjugation (Lederberg et al., 1952). Redundant plasmid exchange between donor cells is prevented by plasmid-encoded proteins that provide exclusion activity by one of two mechanisms (Zechner et al., 2000). Either the formation of stable mating aggregates between two donor cells is inhibited by an outer membrane protein (surface exclusion), or the signal for DNA transfer between donors is blocked by an inner membrane protein after mating pairs have been established (entry exclusion). Some plasmids such as F encode both types of plasmid exclusion mechanisms (Jalajakumari et al., 1987), while only one of the two modes contributes to the exclusion phenotype of ColE1, pKM101, R144, and RP4 (Haase et al., 1996; Hartskeerl et al., 1985; Pohlman et al., 1994; Yamada et al., 1995).

The current study focuses on the entry exclusion properties of conjugative plasmid, pVT745, which was derived from the Gram-negative, periodontal pathogen *Actinobacillus actinomycetemcomitans*

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doi:10.1016/j.plasmid.2005.07.003

(LeBlanc et al., 1993). Plasmid pVT745 is of particular interest in oral microbiology. The presence of pVT745 remnants in the chromosome of various *A. actinomycetemcomitans* strains are suggestive of conjugative gene transfer in the oral cavity (Novak and Leblanc, 1994). Thus, a thorough functional analysis of pVT745 conjugative properties is in order. Previous nucleotide sequence analyses of pVT745 revealed the presence of a gene, *magB05*, that encodes a protein with strong homologies to Eex, a small, outer membrane lipoprotein associated with entry exclusion on pKM101 (Galli et al., 2001; Pohlman et al., 1994). Computer analyses indicated that *magB05* encodes a lipoprotein of 75 amino acid residues. The gene maps in the *magB* gene cluster, which is associated with mating pair formation (Galli et al., 2001). Gene *magB05* appears to be transcriptionally coupled to *magB04* and *magB06* (Fig. 1) (Galli et al., 2001). In this report we confirmed that an entry exclusion function is present on pVT745 and that it requires a functional *magB05* gene.

Actinobacillus actinomycetemcomitans strains VT745 and ATCC29522Rif*recA* (Chen et al., 2002) were grown in TSBYE (3% trypticase soy broth, 0.6% yeast extract) at 37 °C in 10% CO_2. A variant of VT745 without its resident plasmid, pVT745, was obtained as described previously (Chen et al., 2002). In short, strain VT745:pDMG21B (a pVT745-derivative) was passaged in broth culture for 200

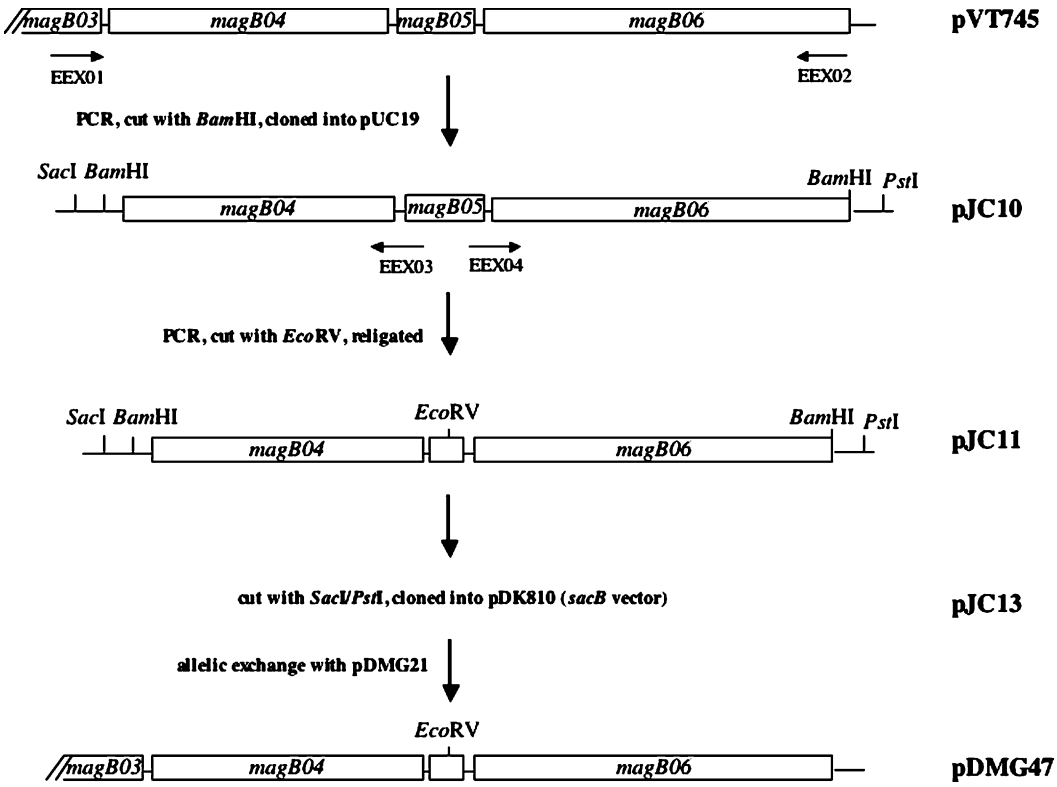

Fig. 1. Construction of pDMG47, a pVT745 subclone with an in-frame deletion in *magB05*. A 2.2-kb fragment containing *magB05* was amplified by PCR with primers EEX01 and EEX02 (both containing a *Bam*HI site) and cloned into pUC19. A second round of PCR with primers EEX03 and EEX04 (both containing an *Eco*RV site) removed a 171-bp fragment within *magB05*. The mutated gene flanked by *magB04* and *magB06* was cloned into pDK810, a vector that contains the counterselectable marker *sacB* (D. Kolodrubetz, personal communication). The recombinant pDK810, pJC13, was introduced via electroporation into *A. actinomycetemcomitans* strain VT745 harboring the pVT745-derivative, pDMG21. Subsequently, wild-type *magB05* on pDMG21 was replaced with the mutated gene through marker exchange-eviction mutagenesis. Boxes represent structural genes. Small horizontal arrows point to the location of primers designed for PCR experiments. DNA fragments are not drawn to scale.