SHORT COMMUNICATION

Introduction of Bacteriophage Mu into *Erwinia stewartii* by Use of an RK2::Mu Hybrid Plasmid

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Although bacteriophage Mu cannot infect *Erwinia stewartii*, lysogens were constructed by conjugal transfer of an RK2::Mu *cts* 62 hybrid plasmid, pRK210, into this species from *Escherichia coli*. The pRK210 transconjugants were stable and produced infectious Mu virions. The Mu *cts* 62 prophage was thermally induced at 42 °C and zygotically induced following transfer of the plasmid to *E. stewartii* recipients. Induction was accompanied by an increased frequency of Gal[−] and Thy[−] mutants among the survivors. Mu promises to be an important tool for studying the genetics of pathogenicity in *E. stewartii*.

INTRODUCTION

Bacteriophage Mu is a useful tool for *in vivo* genetic engineering due to its ability to act as a transposable genetic element (Bukhari, 1976). It can mediate transfer of chromosomal markers by promoting the formation of prime plasmids or the integration of plasmids into the chromosome to form Hfr donor strains. The host range of Mu is normally restricted to certain strains of *Escherichia coli*, *Shigella dysenteriae*, *Citrobacter freundii* and *Klebsiella pneumoniae* (Howe & Bade, 1975). However, the insertion of Mu into broad host range P-1 incompatibility group (IncP-1) plasmids, such as RP4, has allowed the introduction of Mu into nitrogen-fixing strains of *K. pneumoniae*, *Rhizobium meliloti* and *Pseudomonas solanacearum* (Boucher *et al.*, 1977; Dénarié *et al.*, 1977) and *Agrobacterium tumefaciens* (van Vliet *et al.*, 1978).

Erwinia stewartii is an important vascular pathogen of corn and offers an attractive model system for studying the genetics of plant virulence because of its ability to exchange plasmids with *Esch. coli* (Coplin, 1978; Coplin & Rowan, 1979). At present, a means of genetic exchange is not available for *E. stewartii*. This paper describes the introduction of Mu into this species as the first step in developing a plasmid-mediated gene transfer system.

METHODS

Bacterial strains and phage. *Escherichia coli* strain BU8843 [Δ *pro-lac trp-8 strA* Su[−] (Mu c⁺Sam 6014)] containing pRK210 has been described by Figurski *et al.* (1976). Plasmid pRK210 is a derivative of the IncP-1 plasmid RK2 which specifies resistance to carbenicillin, kanamycin and tetracycline, sensitivity to phage PRD1, and contains a Mu *cts* 62 prophage. Plasmid pRK212.1 was derived from pRK210 by deletion of the Mu prophage and genes for kanamycin resistance (Figurski *et al.*, 1976). *Escherichia coli* EG47 *res* (Goldberg *et al.*, 1974) was used to assay Mu phage. Wild-type *E. stewartii* strains SW2, SW3 and SS104 have been described previously (Coplin, 1978).¹DC283 is a nalidixic acid resistant (Nal^r) mutant of SS104 and DC260 is a His[−]Pro[−]Nal^r mutant of SW2. The IncP-specific phage PRD1 (Olsen *et al.*, 1974) was used to verify the presence of pRK210.

Media. L-Broth and L-agar (Luria *et al.*, 1960) were used for most experiments. Thy⁻ mutants were selected on L-agar containing trimethoprim (20 µg ml⁻¹) and thymine (20 µg ml⁻¹). Gal⁻ mutants were selected on DG agar which contained (per litre) 1.0 g NH₄H₂PO₄, 0.2 g KCl, 0.2 g MgSO₄·7H₂O, 20 ml glycerol, 0.1 g yeast extract, 1.0 g 2-deoxy-D-galactose and 15 g agar (pH 7.0).

Plasmid transfer. Matings were performed on nitrocellulose filters at 30 °C for 3 h on L-agar; the donor to recipient ratio was 1:10 (Coplin, 1978). Transconjugants were selected on L-agar containing tetracycline (20 µg ml⁻¹) and nalidixic acid (20 µg ml⁻¹).

Assay for phage. Mu phage were titrated against EG47 using L-broth diluent, L-base agar and L-soft agar overlays containing 1 mM-MgSO₄ and 5 mM-CaCl₂. Plates were incubated at 42 °C.

Pathogenicity tests. Toothpicks were used to inoculate single colonies into 8 d-old sweet corn cv. 'Ear-liking' seedlings by stabbing the pseudostem about 1 cm above the soil line. Seedlings were grown in sterile soil in the greenhouse at 28 °C with supplementary fluorescent lighting. Symptoms were recorded after 10 d.

RESULTS

Transfer of plasmid pRK210 from *Esch. coli* BU8843 into *E. stewartii* DC283 occurred at a frequency of 4×10^{-3} transconjugants per input donor cell. *Erwinia stewartii* SW2 and SW3 also accepted pRK210 at similar frequencies.

The *E. stewartii* strains used in this study are naturally Mu-resistant and do not harbour phages capable of infecting *Esch. coli*. However, supernatants from cultures of the above transconjugants contained phage which formed plaques on EG47 but not EG47 (Mu *cts* 62). Furthermore, phage production by pRK210 transconjugants was thermally inducible. Supernatants from overnight broth cultures of SW3 (pRK210) contained about 10⁵ plaque-forming units ml⁻¹ when grown at 30 °C and about 10⁸ plaque-forming units ml⁻¹ at 37 °C.

Erwinia stewartii does not grow appreciably at 42 °C but can tolerate this temperature for at least 24 h without loss of viability. When SW2 (pRK210) was transferred from 30 to 42 °C, lysis and phage release occurred after 2 h, whereas the parent strain SW2 continued to grow slowly (Fig. 1*a*). To determine the conditions required for full and partial induction of Mu *cts* 62 in *E. stewartii*, broth cultures of SW3 (pRK210) were grown at 30 °C, heated at 42 °C for various times up to 4.5 h and then incubation was continued at either 30 or 37 °C. After 4.5 h total incubation (including the heat treatment period) phage were assayed (Fig. 1*b*). Phage production was maximal when cells were heated for 75 min and then shifted to 37 °C. In contrast, fewer phage were produced and a longer heat treatment was required when the cultures were shifted to 30 °C.

Heat induction of the Mu *cts* 62 prophage increased the frequency of mutants among the survivors. Strains SW2 (pRK210) and SW2 were heated at 42 °C for 2 h and then grown overnight at 30 °C. Survival of SW2 (pRK210) was 10 % following this heat treatment. The average frequency of Thy⁻ mutants per cell was 1.7×10^{-7} for SW2 (pRK210) and 1.6×10^{-8} for SW2. Likewise, the frequency of Gal⁻ mutants per cell was 1.4×10^{-5} for SW2 (pRK210) and 1.9×10^{-7} for SW2. Increases in the number of mutants due to Mu replication (11-fold for Thy⁻ and 74-fold for Gal⁻) were statistically significant ($P = 0.05$). As additional evidence for mutagenesis by Mu, strain DC283 (pRK210) was induced as above and it was found that 5 % (23/450) of the survivors had lost the ability to wilt corn seedlings but still produced necrotic lesions at the site of inoculation and 1 % (2/200) were non-reverting auxotrophs. An equivalent number of clones from DC283 were tested and all were prototrophic and fully virulent. One survivor lost RK2 markers but was still lysogenic for Mu *cts*. Agarose gel electrophoresis of cleared lysates (Meyers *et al.*, 1976) prepared from this strain [DC283 (Mu *cts* 62)] revealed that pRK210 had been lost and the Mu prophage had apparently transposed to the chromosome since there was no increase in the molecular weight of any resident cryptic plasmid (Coplin & Rowan, 1979).

To determine if zygotic induction occurred following transfer of pRK210 into *E. stewartii*,

Fig. 1. Thermal induction of Mu *cts* 62 in *Erwinia stewartii* SW2 (pRK210) and SW3 (pRK210). (a) L-broth cultures were grown at 30 °C and then shifted to 42 °C at the time indicated. Absorbance at 540 nm of SW2 (pRK210) (●) and SW2 (○), and plaque-forming units (□) from SW2 (pRK210) were monitored. (b) L-broth cultures of SW3 (pRK210) were grown at 30 °C, heated at 42 °C for the period indicated and then incubation was continued at either 30 °C (●) or 37 °C (○); after 4.5 h total incubation (including the period at 42 °C) plaque-forming units were assayed.

the frequency of pRK210 transfer from BU8843 (pRK210) into DC283 was compared with that into DC283 (Mu *cts* 62). If zygotic induction occurred, most DC283 recipients would be killed due to induction and lytic replication of the Mu prophage on pRK210. On the other hand, the presence of the Mu prophage in DC283 (Mu *cts* 62) would prevent induction. Since transfer of pRK210 was higher into DC283 (Mu *cts* 62) (1.5×10^{-1} transconjugants per donor cell) than into DC283 (2.3×10^{-3} transconjugants per donor cell) it appears that zygotic induction took place. Additional evidence was obtained by comparing the transfer frequency of pRK210 with that of pRK212.1 which does not contain Mu. In matings between SW3 and DC260, pRK212.1 transferred at 5.5×10^{-6} transconjugants per donor cell, whereas transfer of pRK210 was not detected ($< 10^{-7}$).

DISCUSSION

Although *E. stewartii* is resistant to Mu infection, Mu was readily introduced into several strains by use of the hybrid plasmid pRK210. *Erwinia stewartii* transconjugants stably maintained this plasmid, expressed all plasmid markers, and in most respects behaved as normal Mu *cts* 62 lysogens. Phage, which could form plaques on *Esch. coli* K12 but not on Mu lysogens, were released spontaneously. The prophage was thermally inducible and exhibited the mutagenic properties of Mu. However, the latent period of Mu and the duration of the heat treatment required for induction were longer in *E. stewartii* than in *Esch. coli*. This may be due to the lower optimum temperature (28 to 32 °C) and longer generation time (90 to 120 min) of *E. stewartii*. Transfer of pRK210 appeared to be accompanied by zygotic induction of the prophage, as shown by a decrease in transfer frequency when Mu was located on the RK2 plasmid and an increase in transfer frequency when the recipient was a Mu lysogen.

In previous studies with IncP-1 plasmids (Coplin, 1978), *E. stewartii* strains were found

to be good recipients but poor plasmid donors. Our inability to detect transfer of pRK210 between *E. stewartii* strains is consistent with these findings and indicates that pRK210 will probably not be useful for mobilizing chromosomal markers in *E. stewartii*. However, the recent finding of a cryptic plasmid in SW2, which is capable of derepressed conjugal transfer in SS104 (Coplin & Rowan, 1979), suggests that this plasmid or derivatives of it containing a Mu prophage and transposable drug resistance markers may be useful for obtaining Mu-promoted chromosome transfer in strain DC283 (Mu *cts* 62).

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