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Mobilizable Cloning Vectors Derived from ColD: Their Use in *Erwinia*

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Abstract

We have developed a set of gene cloning vectors derived from the colicinogenic plasmid ColD-CA23. These vectors are mobilizable from *Escherichia coli* to *Erwinia chrysanthemi*. Transformation of *Erwinia*, can therefore be circumvented. With these vectors, the initial cloning, plasmid auto-amplification and DNA extraction can be performed easily in *E. coli* using standard methods. Manipulation and mutagenesis of the cloned genes are then possible, and subsequent mobilization of the hybrid plasmids from *E. coli* to *Erwinia* for the expression and analysis of the cloned genes is highly efficient.

Keywords: Recombinant DNA, *Erwinia*, mobilization, plasmid vector

Abbreviations: Ap: Ampicillin; kb: kilobase pair; Km: Kanamycin; Sm: Streptomycin; Spc: Spectinomycin; Tc: Tetracycline

1. Introduction

The efficiency of transformation of the phytopathogenic bacteria *Erwinia* is very low (Reverchon and Robert-Baudouy, 1985). This makes gene cloning and screening of gene libraries in these bacteria very difficult. A possibility to circumvent transformation in these bacteria is to perform the initial cloning in known strains of *Escherichia coli* which have the advantage to be highly transformable and to be deficient in restriction and recombination, and then to introduce the hybrid plasmids into *Erwinia* by conjugative mobilization using a "helper" plasmid. The colicinogenic plasmid ColD-CA23 (Timmis, 1972; Frey et al., 1986) is a small, 5.12 kb, high copy number plasmid which

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can be mobilized efficiently with helper plasmids from the incompatibility group Inc FII such as RTF-Tc (Frey et al., 1986). ColD and its derivatives have been shown to autoamplify and reach a considerable high copy number in stationary phase of growth in *E. coli* (Frey and Timmis, 1985). We have used the region of ColD which contains the replication and mobilization functions and various antibiotic resistance genes to construct gene cloning vectors. Each of the vectors contains a "cassette" of two antibiotic resistance genes, separated on each side by strong transcriptional stop signals to prevent transcription out of the cloning "cassette" into the genes necessary for stable plasmid replication and mobilization. These vectors are mobilizable from *E. coli* to *Erwinia chrysanthemi*, are readily selectable in both species and provide therefore a useful tool for gene cloning experiments in *Erwinia*.

2. Materials and Methods

The bacterial strains used in this study were the following: *E. coli* K12 C600, *thr leu thi lac* (Bachmann, 1972) and *Erwinia chrysanthemi* A555 *arg met lac* (obtained from J. Robert-Baudouy). Plasmids ColD-CA23 (Timmis 1978; Frey et al., 1986), pKT235 (Frey and Timmis, 1985) pHP45-omega (Prentki and Krisch, 1984; Frey and Krisch, 1985), and RTF-Tc (a R100.1 derivative) (Cohen and Miller, 1970) have been described previously.

Procedures for the purification of plasmid DNA, cleavage by restriction endonucleases, gel electrophoresis, gene cloning and transformation of bacteria have been described by Maniatis et al. (1982). Mobilization was performed as described (Bagdasarian et al., 1981).

3. Results

The *EcoRV* — *HincII* fragment of ColD, which contains the function for autonomous replication and mobilization (Frey et al., 1986) (see Fig. 1) was ligated to the *HindIII* fragment of pHP45-omega which contains the Streptomycin/Spectinomycin resistance gene (Sm/Spc) and the flanking transcriptional stop signals (Fig. 1) by first "filling in" the *HindIII* sites using Klenow enzyme, in order to create "blunt ends". This construction resulted in plasmid pJFF12 (Fig. 1). The gene cloning vectors were then constructed by ligating the *HaeII* fragments of pKT235 containing the antibiotic resistance genes for Kanamycin (Km), and Ampicillin (Ap), into the *SphI* site of pJFF12 after making the restriction fragments blunt ended using Klenow enzyme. These constructions resulted in the cloning vectors pJFF15 (Sm/Spc; Km) and pJFF16 (Sm/Spc; Ap) as shown in Fig. 1. The relative

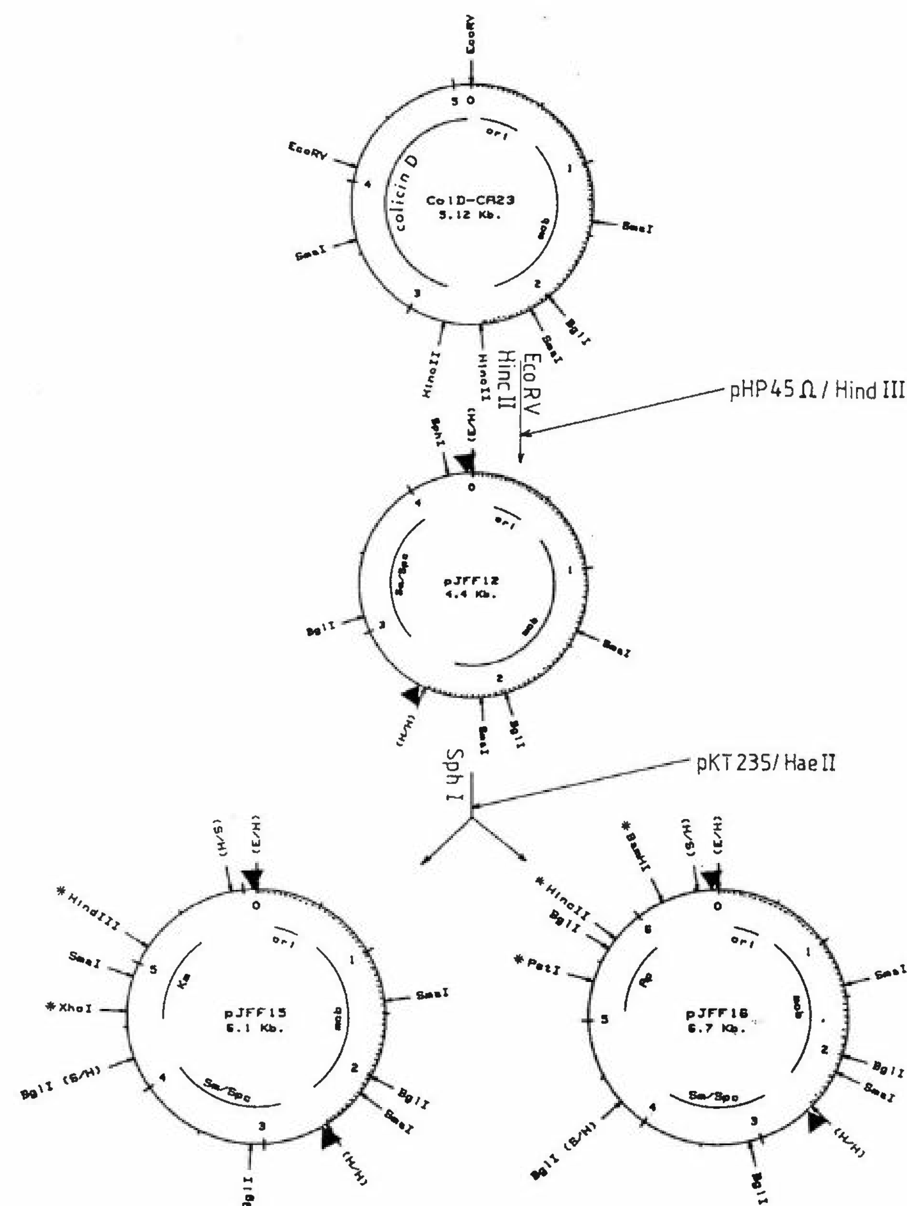

Figure 1. Construction of the vectors pJFF15 and pJFF16. Dotted lines represent the segment from ColD. The filled triangles indicate the position of the transcription stop signals from pHP45-omega. "Ori" indicates the position of the origin of replication and "mob" the genes for mobilization. Details of the construction are described in the text.

orientation of the inserted fragments were confirmed by digestions of various combinations of restriction enzymes.

The plasmids pJFF12, pJFF15 and pJFF16 were transformed into *E. coli* strain C600 containing plasmid RTF-Tc (Cohen and Miller, 1970) which serves as "helper" plasmid for the subsequent mobilizations. The resulting strains were grown to late exponential phase, mixed with an equal amount of a culture of *Erwinia chrysanthemi* A555 in late exponential phase and used for filter matings. Selection for *Erwinia chrysanthemi* clones containing the various cloning vectors were made on minimal plates supplemented with arginine and methionine (for counterselection of *E. coli* C600) and the appropriate antibiotic for selection of the vector. The presence of the vectors in *Erwinia chrysanthemi* was controlled by testing for the second antibiotic resistance and by extraction of plasmid DNA. Mobilization frequencies obtained varied between 0.1 and 0.01.

4. Discussion

We have shown that the replicon of ColD is able to replicate and to be stably maintained in *Erwinia chrysanthemi*. Previous attempts failed to establish the ColD derived vectors pKT235 and pKT252 (Frey and Timmis, 1985) in *Erwinia chrysanthemi* by transformation; the strain produced very small colonies on plates and grew very slowly after introduction of the plasmids (results not shown). We attribute this behaviour to the fact that the vectors pKT235 and pKT252 contained the entire SOS inducible colicin operon, since all other ColD derived plasmids which are devoid of a functional colicin operon are able to establish stably in *Erwinia chrysanthemi* (results not shown). We have therefore used the region of ColD containing only the necessary replication and mobilization functions in order to construct the cloning vectors pJFF15 and pJFF16. Both plasmids contain the Sm/Spc resistance gene from the Omega fragment which was shown to be easily selectable in *Erwinia chrysanthemi* A555 (Frey and Krisch, 1985). Plasmid pJFF 15 contains in addition the Km resistance gene with the single restriction sites *HindIII* and *XhoI* which are useful for cloning of DNA fragments generated by these enzymes, and for screening of colonies which have hybrid plasmids by the insertional activation of the Km resistance. Plasmid pJFF16 contains the Ap resistance gene with the restriction sites *PstI, HincII* (for cloning of "blunt end" fragments) and *BamHI* as cloning sites using the Ap gene for screening by insertional inactivation. Both plasmids are mobilizable from *E. coli* to *Erwinia* and can therefore be used as shuttle vectors for gene cloning into *Erwinia*.

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