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(Short Communication)

Xanthomonas plasmid pXW45N replicates in *Escherichia coli.*

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Abstract. A DNA fragment essential for the replication of the *Xanthomonas* plasmid pXW45N has been isolated and analyzed in *Escherichia coli.* At least two regions within the fragment are necessary for the autonomous replication of this plasmid in *E. coli.* This plasmid is compatible to pBR322, pACYC184, RSF1010 and RP4 and may be a broad-host-range plasmid.

Key words: Autonomous replication; *Escherichia coli*; *Xanthomonas* plasmid.

Introduction

Strains of *Xanthomonas campestris* pv. *citri* are Gram-negative phytopathogenic bacteria responsible for citrus canker. In *X. campestris* pv. *citri* XW45, two cryptic plasmids, pXW45N and pXW45J, have been identified. They are 64 and 35 Kb in size, respectively. Two *Xanthomonas* transposable elements ISXC*4* and IS*XC5* which originate independently from pXW45N and pXW45J were isolated from XW45 (Tu *et al.*, 1989a). The elements share DNA homology and are capable of transposition in *Escherichia coli*. Besides the transposable elements, functions of the plasmids still remained unknown. However, in our recent study, we found that one of the replicons, pXW45N, could be stably maintained in *E. coli*. At least two regions in the plasmid were necessary for its autonomous replication. For investigating the DNA fragments of *Xanthomonas* plasmids essential for replication, a strategy of growing drug-resistant derivatives of the plasmids in *E. coli* was used. Fragments of *Xanthomonas* plasmid were ligated to a Km^r fragment of Tn903 and allowed to transform *E. coli.* Because a functional replication origin (*oriV*) was absent in the Km^r fragment, stable Km^r plasmids containing fragments of *Xanthomonas* plasmid could be replicons using *oriV* of the *Xanthomonas* plasmids.

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Materials and Methods

Bacterial strains and plasmids used are listed in Table 1. Enzymes were from Boehringer Mannheim GmbH. Conditions for enzyme reaction were as described by the manufacturer. The calcium shock method of Dagert and Ehrlich (1979) was used for *E. coli* transformation.

Results and Discussion

To achieve a small Km^r fragment flanked by two *Asp718* sites (*Asp718* is an isoschizomer of *KpnI*, but creates 5'-protruding termini), plasmid pMZ1 was first constructed by religating the *HindII* partial digest of pML21 which carries the replication origin of ColE1 (Hershfield *et al.*, 1976) (Fig. 1). The 3.4-Kb *Asp718* fragment containing the Km^r gene of pMZ1 was then isolated, dephosphorylated and ligated to the *Asp718* digest of *Xanthomonas* plasmids pXW45N and pXW45J (Tu *et al.*, 1989a). After transforming *E. coli*

Table 1. *Bacterial and plasmids strains*

Strain	Relevant characteristics[a]	Source or reference
Escherichia coli DH1	F⁻*rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17 (r_k^-, m_k^+)	Hanahan, 1983
Xanthomonas campestris		
pv. *citri* XW45	Wild type	Wu *et al.*, 1986
Plasmid		
pXW45N, pXW45J	Cryptic	Tu *et al.*, 1989a
pML21	Km^r	Hershfield *et al.*, 1976
pMZ1	Km^r	This work
pXWMZ3	Km^r	This work
pXWMZ3HD	Km^r, *HindIII* deletion mutant of pXWMZ3	This work
pXWMZ3SD	Km^r, *SalI* deletion mutant of pXWMZ3	This work
pXWMZ3HSD	Km^r, *HindIII*-*SalI* double deletion mutant of pXWMZ3	This work
pBR322	Tc^r Ap^r	Bolivar *et al.*, 1977
pACYC184	Tc^r Cm^r	Chang and Cohen, 1978
RSF1010	Su^r Sm^r	Guerry *et al.*, 1974
RP4Δ7	Ap^r Tc^r	Tu *et al.*, 1989b

[a] Cm, chloramphenicol; Tc, tetracycline; Km, kanamycin; Sm, streptomycin; Ap, ampicillin; Su, sulfonamides.

DH1, Km^r transformants were screened (Tu and Charng, 1989). The results showed that only the plasmids containing a 29-Kb *Asp718* fragment of *Xanthomonas* plasmids could be obtained. The *Asp718* fragment has been located between the coordinates 63 and 28 in the map of pXW45N according to physical mapping and DNA hybridization. The Km^r gene could be inserted into the *Xanthomonas* replicon in both orientations. The resulting derivatives were designated pXWMZ2 and pXWMZ3, respectively. The restriction endonuclease cleavage map of pXWMZ3 is shown in Fig. 1.

To detecting the regions essential for the autonomous replication of pXWMZ3 in *E. coli*, deletion analysis has been performed by recircularizing independently the *BglII, HindIII, HpaI, SalI* and *XhoI* fragments of pXWMZ3. Km^r DH1 transformants were analyzed. The results are summarized in Table 2. In pXWMZ3 the *HindIII* fragment from coordinate 12.5 to 21.7 and the *SalI* fragment from 30.1 to 4.7 appeared unnecessary for the maintenance of the plasmid, since *HindIII* and *SalI* deletion mutants and *HindIII* and *SalI* double deletion mutants could be isolated (Fig. 2). The DNA fragment from the *SalI* site at coordinate 4.7 to the *HindIII* site at 12.5 and the fragment from the *HindIII* site at about 21.7 to the *Asp718* site at 27.9 were not deletable. According the results, we suggest that the *oriV* of pXW45N may lay in one of these two regions. Gene(s) other than *oriV* may be also important for the maintenance and regulation of pXWMZ3 in *E. coli.*

Table 2. *Deletion analysis of pXWMZ3 fragments*

Enzyme	Coordinate	Result[a]
BglII	6.7−18.8	−
HindIII	12.5−21.7	+
HpaI	26.0−28.1	−
SalI	30.1− 4.7	+
XhoI	15.4−27.4	−

[a] + = deletable; − = not deletable.

To develop pXWMZ3 into a cloning vector, stability of pXWMZ3 and compatibility of pXWMZ3 with a

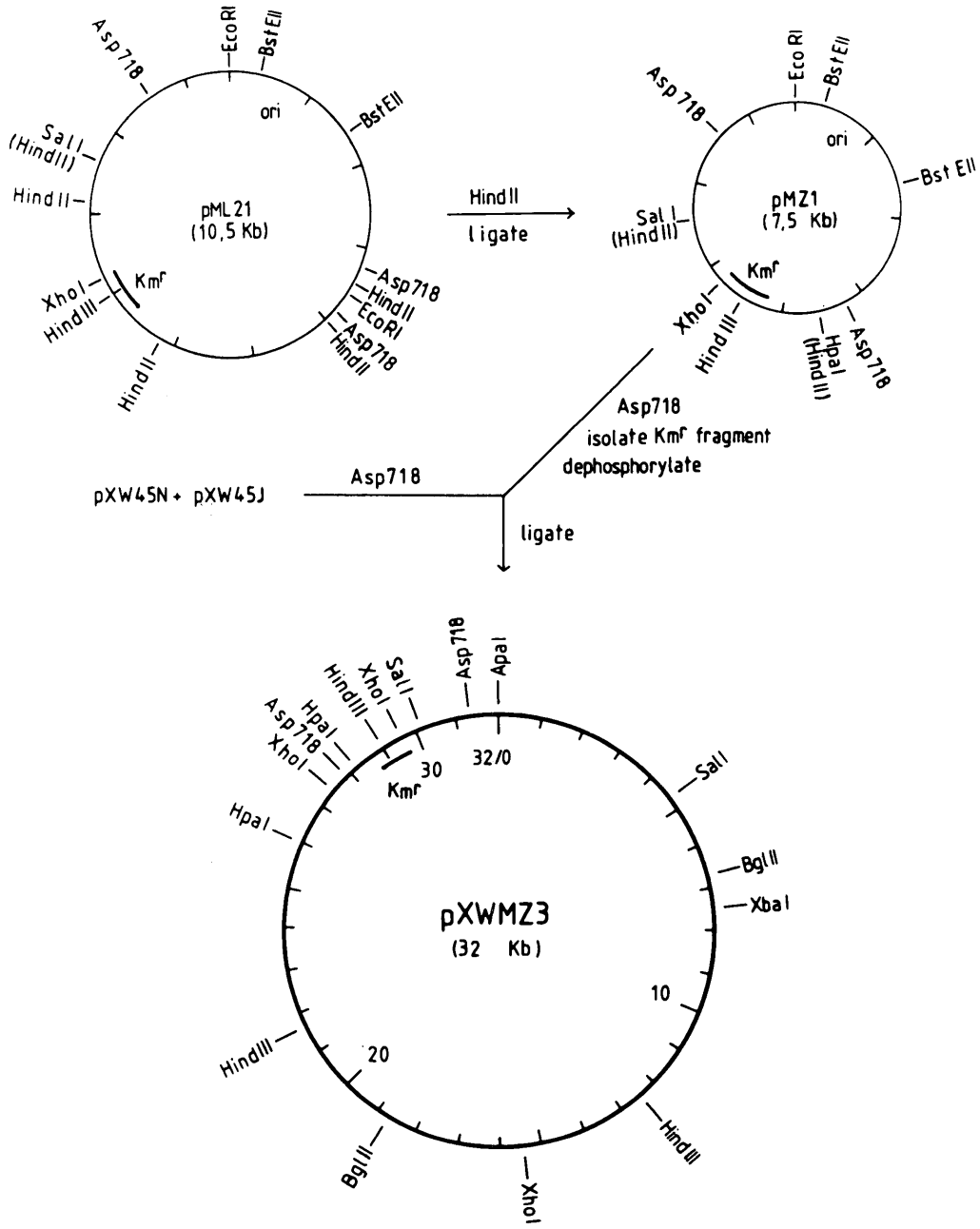

Fig. 1. Construction and physical map of pXWMZ3. Km^r refers to kanamycin resistance. pXW45N and pXW45J were isolated from *X. campestris* pv. *citri* XW45 as plasmid mixture. pXWMZ3 is about 32.0 Kb in size; its Km^r *Asp718* fragment between coordinate 27.9 and 31.3 was from pMZ1, the remaining part from pXW45N.

commonly used cloning vector pBR322 and pACYC184, and broad-host-range plasmids RSF1010 and RP4 (Datta *et al.*, 1971) were examined. Cultures of DH1 which contained pXWMZ3 and one of pBR322, pACYC184, RSF1010 or RP4Δ7 (Tu *et al.*, 1989b) were grown under the antibiotic selection to force the maintenance of both plasmids. The cultures were then diluted into LB media selecting only for pBR322, pACYC184, RSF1010 or RP4Δ7 and allowed to grow at 37°C for 10 generations. Appropriate dilutions were plated on LB agar to obtain single colonies and the drug resistance phenotype of the colonies were

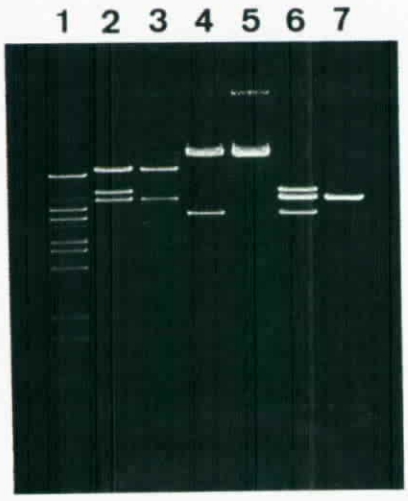

Fig. 2. Digestion patterns of pXWMZ3 and its derivatives. (1) λ/*BstEII*, (2) pXWMZ3/*HindIII*, (3) pXWMZ3HD/*HindIII*, (4) pXWMZ3/*SalI*, (5) pXWMZ3SD/*SalI*, (6) pXWMZ3/*HindIII*-*SalI*, and (7) pXWMZ3HSD/*HindIII*-*SalI*.

examined. After 10 generations of growth, 83% of the cells still harbored pXWMZ3 both in the presence or absence of a second plasmid. The results suggested that pXWMZ3 is compatible with those plasmids.

The results described above demonstrate that pXW45N is a useful plasmid. The ability of pXW45N to replicate in both *E. coli* and *X. campestris* pv. *citri* suggested that it is probably a broad-host-range plasmid and may potentially be developed to a cloning vector for the analysis of *Xanthomonas* genes. On the other hand, deletion analysis and subcloning of various regions of pXWMZ3 allow the investigation of the replication mechanism of pXW45N in *E. coli*.

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柑橘潰瘍病菌質粒 pXW45N 在大腸桿菌中複製

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以 DNA 重組技術將不含 DNA 複製起點之抗抗黴素(kanamycin)基因與柑橘潰瘍病菌質粒 pXW45N 之 DNA 片段相連後轉形大腸桿菌。在大腸桿菌中分析得知一長度約 29 Kb 之選殖片段對 pXW45N 的複製相當重要。這段 DNA 內至少有兩個區域爲複製所需。含此 DNA 片段之質粒能與 pBR322，pACYC184，RSF1010 和 RP4 相容。pXW45N 因此可能是泛宿主性質粒。