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[Short Communication]

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Construction of a High-efficiency Shuttle Vector Containing the Minimal Replication Origin of *Bacillus thuringiensis*

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In order to improve the transformation efficiency of the *Bacillus thuringiensis* (Bt)-*Escherichia coli* (*E.coli*) shuttle vector, pHT3101, we intended to minimize replication origin of Bt in pHT3101. For this, two modified shuttle vectors, pHT1K and pHT261, in which 2.9 kb of replication origin of Bt were shortened to 1 kb and 261 bp, respectively as previously reported. Whereas the pHT1K could efficiently transform Bt into the antibiotic resistant, no transformants were obtained with pHT261. Furthermore, pHT1K showed higher transformation efficiency compared to that of parent vector, pHT3101. Therefore, pHT1K might be a very useful Bt-*E. coli* shuttle vector carrying minimal replication origin of Bt.

Key words: *Bacillus thuringiensis*, Shuttle vector, Replication origin, pHT1K

Introduction

The gram-positive spore-forming *Bacillus thuringiensis* is well known for its ability to produce insecticidal crystalline inclusion bodies during sporulation. These inclusions are consisted with more than one type of insecticidal crystal proteins (ICPs) (Höfte and Whiteley, 1989; Schnepf *et al.*, 1998) and have been used as one of the most successful biological control agents for suppression of agriculturally and medically important insect pests (Kronstad *et al.*, 1983).

In addition, an efficient transformation systems for *B. thuringiensis* have been developed to manipulate the production, regulation, and activity of ICPs by molecular genetic techniques and to construct improved *B. thuringiensis* strains for use as microbial pesticides (Miteva and Grigorova, 1988). The success of this approach would depend on the availability of high-efficient cloning vectors, and these experiments were performed using a convenient shuttle vector, pHT3101 constructed from the *B. thuringiensis* resident plasmid pHT1030 previously characterized for its segregational stability (Lereclus *et al.*, 1988). This chimeric plasmid allows the cloning of large DNA fragments which are stably maintained both in *B. subtilis* and *B. thuringiensis* (Lereclus *et al.*, 1989). However, the efficiency of transformation would depend on the introduced DNA size. In general, transformation efficiency tends to go down as the size of the DNA being transformed goes up (Baum *et al.*, 1990; Baum and Gilbert, 1991; Siguret *et al.*, 1994; Mesrati *et al.*, 2005). Therefore, constructing a small size vector that could efficiently transform *B. thuringiensis* strains is valuable.

In this study, we focused on minimizing the 2.9 kb of replication origin (*ori*) of *B. thuringiensis* within pHT3101 to improve the transformation efficiency of this vector (Lereclus and Arantes, 1992).

Materials and Methods

About 6.4 kb fragment PCR amplified from the pHT3101 using oligonucleotide primers, SF (5'-CGAATTCGAGC TCGGTACCCG-3') and SR (5'-CACTCAAAGGCGGT

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AATACGG-3'), was re-circularized by self-ligation, to obtain pHT3101-S. The pHT261 was constructed by PCR amplification of about 3.3 kb fragment from the pHT3101-S with BTSV-261F (5'-CGTTTTTGAACACCACGATATC TA-3') and BTSV-MCSR (5'-GGGTTTTCCCAGTCAC-GACGTTG-3') primers and re-circularization of this fragment. Also, the pHT1K was constructed by self-ligation of about 4.7 kb of PCR amplified fragment using oligonucleotide primers, BTSV-EmrF (5'-TCCCCTTAGAAG-CAAACTTAAGAGTG-3') and BTSV-1KoriR (5'-TCTG GTAGTTGCTTCAGTACTCTACA-3') templated with pHT3101-S. Internal structure of the constructed vectors, pHT261 and pHT1K, were confirmed with restriction endonuclease digestion and nucleotide sequence determination (Arantes and Lereclus, 1991).

The pHT3101 and the two constructed vectors, pHT261 and pHT1K, were transformed into acrystalliferous *B. thuringiensis* subsp. *kurstaki* Cry⁻B (Cry⁻B) strain by electroporation using Gene Pulser II (BIO-RAD, USA) at 25 uF, 1.8 kV. The Pulse Controller Plus (BIO-RAD, USA) was set to 400 Ω with 0.2 cm cuvettes (BIO-RAD, USA). The transformed Cry⁻B cells were plated on nutrient agar plate containing erythromycin (Em, 25 μg/ml). After incubation at 30°C for 1 day, Em resistant transformant colonies were counted to compare transformation efficiency.

Results and Discussion

Previously, two regions of pHT3101, 261 bp and 1 kb, were reported as minimal *ori* of *B. thuringiensis* in pHT3101, respectively (Lereclus and Arantes, 1992; Yu *et al.*, 2000). To confirm the true minimal *B. thuringiensis ori*, pHT261 and pHT1K, in which 261 bp and 1kb region were contained as minimal *B. thuringiensis ori*, respectively, were constructed (Fig. 1).

In electroporation of pHT261 using three different DNA quantities (100, 200 and 400 ng, respectively), no transformant colonies resistant to Em were obtained. However, in case of pHT1K, Em resistant transformant colonies were obtained with 5.1~13.3 times higher efficiency than that of parental vector, pHT3101 (Table 1). These results suggest that the true minimal *ori* of *B. thurigiensis* in pHT3101 is the 1 kb region reported by Lereclus *et al.* (1992) and the 261 bp region reported by Yu *et al.* (2000) does not function as *ori* in *B. thuringiensis* cells. In addition, the size of transformed DNA was a critical factor for efficiency of transformation because the smaller vector, pHT1K showed higher efficiency, pHT3101.

In conclusion, the newly constructed pHT1K carrying the true minimal replication origin could be a useful *B. thuringiensis-E. coli* shuttle vector for efficient transformation of *B. thuringiensis* strains.

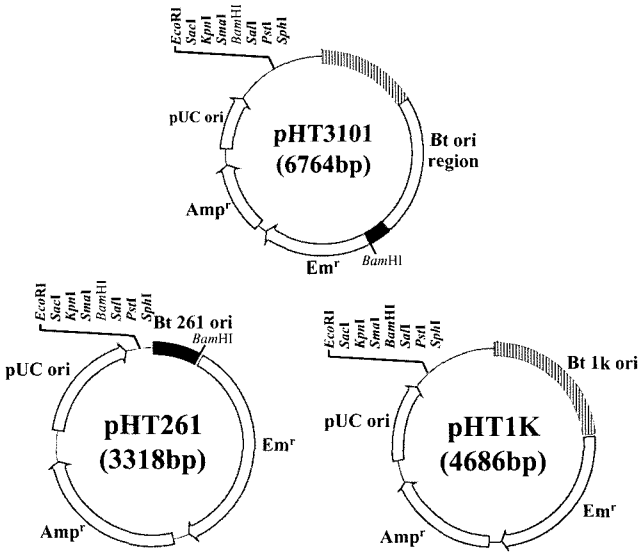

Fig. 1. Circular map of *B. thuringiensis-E. coli* shuttle vectors, pHT3101, pHT261 and pHT1K. The black solid and striped boxes indicated previously reported minimal replication origin of 261 bp and 1 kb, respectively. pUC *ori*, replication origin of *E. coli*; Em^r, erythromycin resistance gene; Amp^r, ampicillin resistance gene.

Table 1. Transformation efficiency of *B. thuringiensis-E. coli* shuttle vectors, pHT3101, pHT261 and pHT1K into acrystalliferous *B. thuringiensis* subsp. kurstaki Cry⁻B

Vector used for electroporation	Quantity of electroporated DNA (ng)	Number of transformant colonies resistant to erythromycin
pHT3101	100	40.0 ± 32.2 cd*
	200	116.3 ± 24.0 b
	400	342.0 ± 173.3 a
pHT261	100	No transformant
	200	No transformant
	400	No transformant
pTH1K	100	3.0 ± 2.6 d
	200	9.3 ± 4.0 d
	400	66.7 ± 28.9 c

*Different letters behind means indicate significant difference ($n = 4$, $p < 0.05$, Duncan's multiple range test).

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