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Characterization of *Nocardia* Plasmid pXT107

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Abstract *Nocardia*, *Rhodococcus* and *Streptomyces*, all members of the actinomycetes family, are Gram-positive eubacteria with high G+C content and able to form mycelium. We report here a newly identified plasmid pXT107 of *Nocardia* sp. 107, one of the smallest circular plasmids found in *Nocardia*. The complete nucleotide sequence of pXT107 consisted of 4335 bp with 65% G+C content, and encoded one replication extragenic palindromic (Rep) and six hypothetical proteins. The Rep, double-strand origin and single-strand origin of pXT107 resembled those of typical rolling-circle-replication plasmids, such as pNI100 of *Nocardia*, pRE8424 of *Rhodococcus* and pIJ101 of *Streptomyces*. The *Escherichia coli-Nocardia* shuttle plasmid pHAQ22, containing the *rep* gene of pXT107, is able to propagate in *Nocardia* but not in *Streptomyces*.

Key words *Nocardia*; plasmid; complete nucleotide sequence; replication

The genera of *Nocardia* and its close relative *Rhodococcus*, belonging to nocardioform actinomycetes, are high G+C Gram-positive eubacteria with fragmentation of mycelium. Many *Nocardia* species, even clinical isolates, can produce bioactive molecules such as antibiotics [1,2] and enzymes of industrial importance [3], whereas some species cause human and animal diseases of lung and brain [4]. Indigenous circular plasmids have been detected among *Nocardia* species [5–7], but few are characterized. The replication extragenic palindromic (Rep) protein of plasmid pNI100 of *Nocardia italica* resembles that of the rolling-circle-replication (RCR) plasmid pSG5 of *Streptomyces*, and a shuttle vector of pNI100 derivative can propagate in *Streptomyces* [8].

Nocardia sp. 107 was identified from a soil sample isolated in Sichuan province, China [9]. We report here the identification, sequencing and characterization of the indigenous plasmid pXT107 from *Nocardia* sp. 107. An *Escherichia coli-Nocardia* shuttle plasmid, containing the *rep* gene, can propagate in *Nocardia* but not in *Streptomyces*.

Materials and Methods

Bacterial strains and plasmids

The list of plasmids and strains used in this work is given in **Table 1**. *Nocardia corallina* 4.1037 was purchased from the Chinese General Microbiological Culture Collection Center (Beijing, China).

Growth conditions, transformation procedures and DNA manipulations

Nocardia strain was grown at 28 ºC in Tryptone Soy Broth media. Plasmid DNA was isolated using both non-alkaline denatured [10] and denatured/renatured procedures [11]. Electroporation of *N. corallina* 4.1037 was done by the method of Yao *et al*. [12]. *Streptomyces* culture, protoplast preparation and transformation were carried out by the method of Kieser *et al*. [11]. The protocol of Sambrook *et al*. [13] was used in *E. coli* DNA manipulations. The 16S rDNA fragment amplification was done by using the actinomycetes-specific 16S rDNA primer pair (16S-F, 5′-

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Table 1 Bacteria strains and plasmids used in this study

Strain or plasmid	Genotype or phenotype	Source or reference
Nocardia sp. 107	Host strain contains plasmid pXT107	This work
Streptomyces lividans ZX7	*pro-2 str-6 rec-46* Δ*dndA* SLP2$^-$ SLP3$^-$	[27]
Nocardia corallina 4.1037	*Nocardia* strain free of plasmid	Stocked in our laboratory
Escherichia coli DH5α	*supE*44 Δ*lacU*169 (phi80 *lacZ* ΔM15) *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1	Gibco-BRL (Grand Island, USA)
pBluescriptII KS (+)	3.0 kb, *bla lacZ*	Stratagene (La Jolla, USA)
pXT107	4.3 kb cryptic plasmid from *Nocardia* sp. 107	This work
pQC156	5.0 kb, *bla tsr mel*	[26]
pTQ104	4.3 kb *Bam*HI fragment of pXT107 cloned into pBluescript KSII (+)	This work
pHAQ20	4.3 kb *Bam*HI fragment of pXT107 cloned into pQC156	This work
pHAQ22	4.3 kb *Pst*I fragment of pXT107 cloned into pQC156	This work
pIJ702	pIJ101 derivative, *tsr mel ltz*$^-$ *sti*$^-$	[28]

AGAGTTTGATCCTGGCTCAG-3′; 16S-R, 5′-TACGGCTACCTTGTTACGACTT-3′) and high fidelity thermostable DNA polymerase.

DNA sequencing and analysis

Plasmid sequence was determined using "primer walking" method from both strands on the ABI 3730 automated DNA sequencer (Applied Biosystems, Foster, USA) at the Chinese Human Genome Center (Shanghai, China). Sequence analysis was carried out with FramePlot 3.0beta software (http://watson.nih.go.jp/~jun/cgi-bin/frameplot-3.0b.pl) [14]. Sequence comparisons were done with software from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). DNA secondary structure was predicted using "mfold" software (http://www.bioinfo.rpi.edu/applications/mfold/old/dna/forml.cgi) [15]. The GenBank accession No. of pXT107 sequence is DQ399903.

Results and Discussion

Identification and complete nucleotide sequencing of plasmid pXT107

The 16s rDNA fragment of strain 107 was amplified by polymerase chain reaction and sequenced. The sequence was searched for similarity in the National Center for Biotechnology Information database by BLASTN and displayed high homology with that of many *Nocardia* strains, such as *Nocardia* sp. DSM 43576 (identity 99%) and *Nocardia flavorosea* JCM 3342 (99%). Circular plasmid DNA was isolated from *Nocardia* sp. 107 using both non-alkaline denatured [10] and denatured/renatured procedures [11], and electrophoresed in an agarose gel. A 4.5 kb DNA band (designated pXT107), resistant to alkaline treatment, was detected (data not shown). Treatment with restriction endonucleases showed that pXT107 contained unique sites of *Bam*HI, *Pst*I, *Xba*I and *Xho*I (**Fig. 1**). The *Bam*HI-digested DNA was cloned into *E. coli* plasmid pBluescript II KS (+) to yield pTQ104. Polymerase chain reaction sequencing of pTQ104 was carried out using "primer walking" (see "Materials and Methods"). The complete nucleotide sequence of pXT107 on pTQ104 consisted of 4335 bp. The G+C content was 65%, resembling that of typical *Nocardia* plasmids (e.g., 67% for pNF1 and 68%

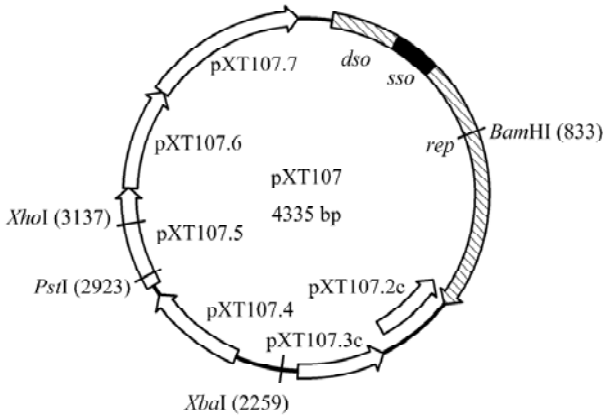

Fig. 1 Physical map and genetic organization of *Nocardia* plasmid pXT107

The unique sites of restriction enzymes were shown on the plasmid. Transcriptions of predicted open reading frames (pXT107.1–7) are indicated by arrowed boxes, pXT107.1 is indicated with *rep*. The double-strand origin (*dso*) and single-strand origin (*sso*) within the *rep* gene are shown.

for pNF2) [16]. The putative open reading frame analysis with FramePlot 3.0beta predicted seven protein-encoding regions, including one Rep and six hypothetical proteins.

Rep, double-strand origin (*dso*) and single-strand origin (*sso*) of pXT107 resemble those of typical RCR plasmids

All RCR plasmids contain three elements: a gene encoding the initiator protein (Rep), the *dso*, and the *sso* [17]. The predicted Rep of pXT107 contained conserved protein motifs I–III [18,19], resembling several RCR plasmids, such as pRE8424 of *Rhodococcus erythropolis*, pIJ101 of *Streptomyces lividans*, pNI100 of *N. italica* and pBL1 of *Brevibacterium lactofermentum* [**Fig. 2(A)**]. The experimentally identified *dso* sequences of *Streptomyces* RCR plasmids pIJ101, pJV1, pSNA1, pBL1 and pSN22 were

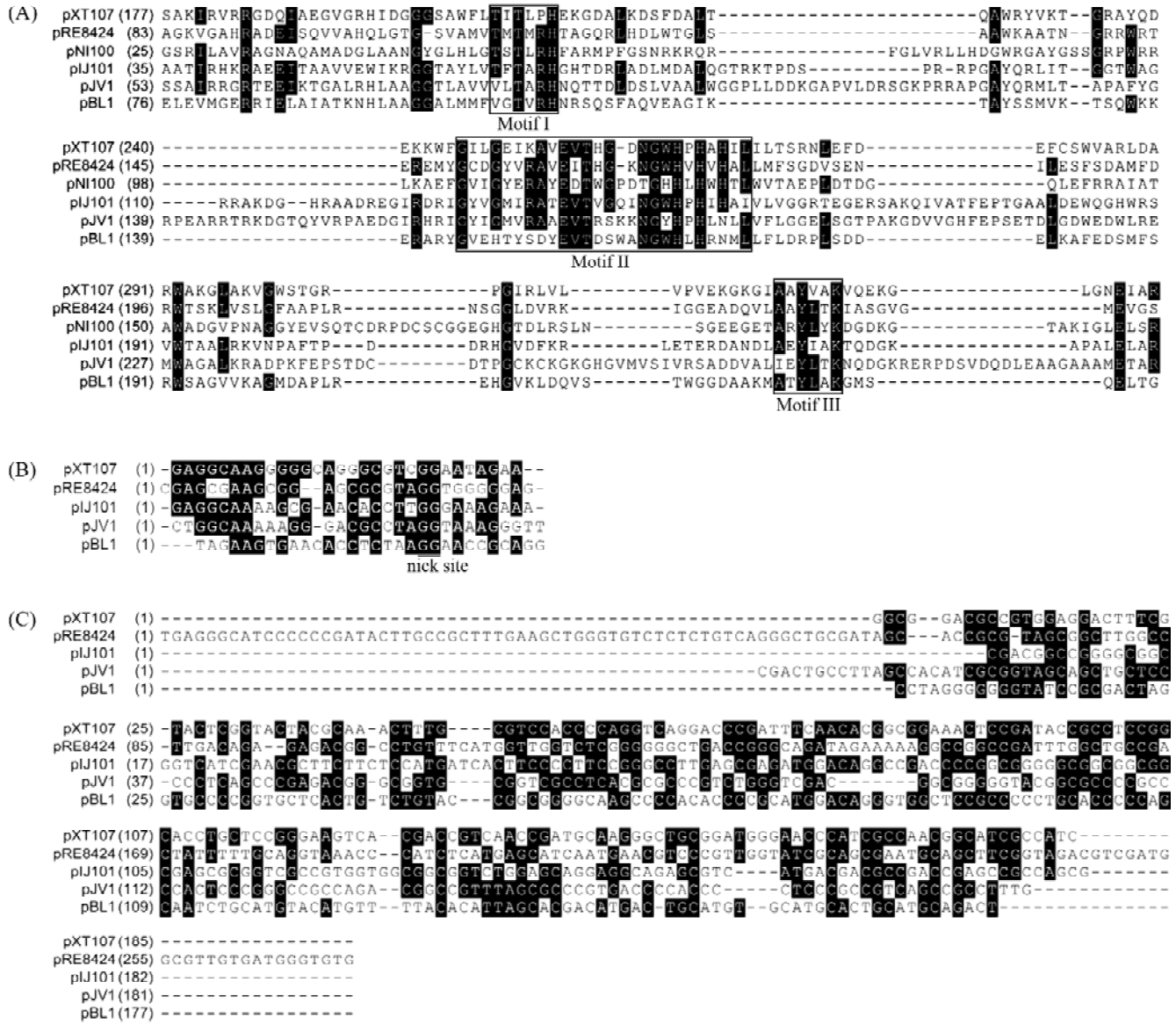

Fig. 2 Comparisons of the replication extragenic palindromic (Rep) proteins, double-strand origin (*dso*) and single-strand origin (*sso*) of rolling-circle-replication plasmids and *Nocardia* plasmid pXT107

(A–C) Alignment of the pXT107 sequence with the known Rep proteins, *dso* and *sso* of plasmids pRE8424, pIJ101, pJV1, pNI100 and pBL1. Three or more identical protein sequences were indicated as blocked. Plasmids pSNA1 and pSN22 also belong to *Streptomyces* as pIJ101 and pJV1, so these two plasmids are not included in **Fig. 2**.

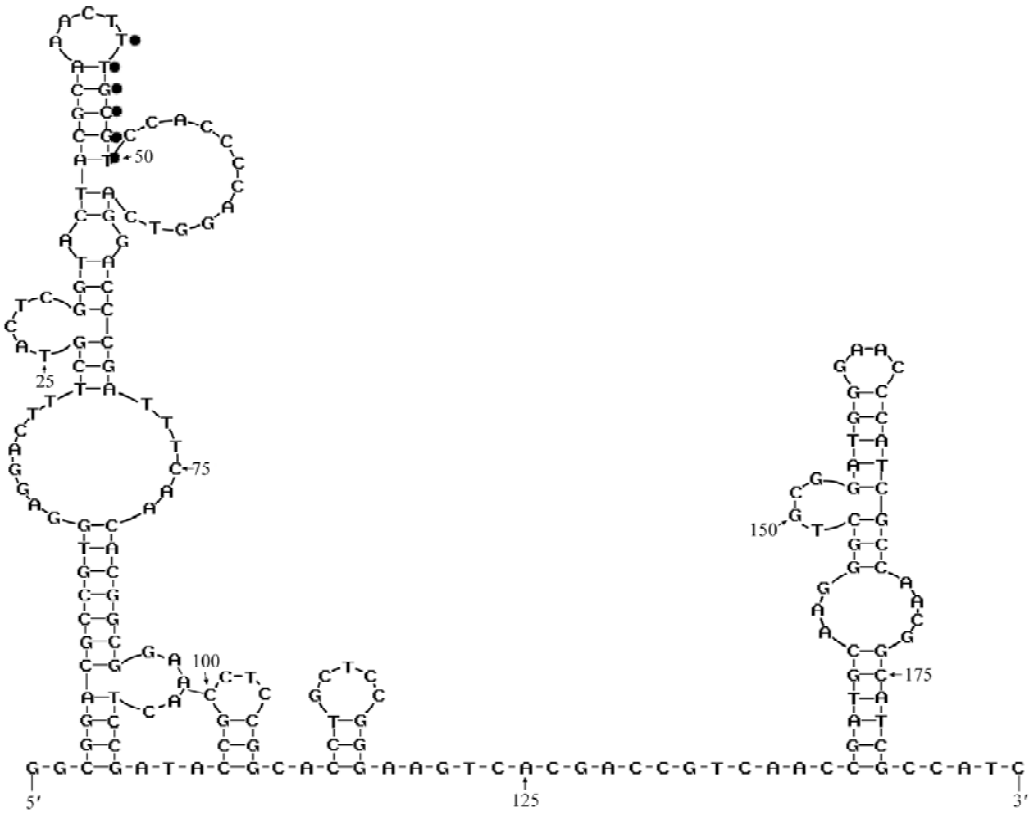

Fig. 3 Predicted secondary structure of the *sso* of pXT107

conserved, especially the GG dinucleotide at the nick site [**Fig. 2(B)**] [20–23]. A similar sequence was also found within the *rep* of pXT107. The *sso* is required for initiation of lagging strand synthesis [17]. Alignment of the pXT107 sequence to the characterized *sso* sequences of pIJ101, pSN22, pBL1 and pRE8424 [21–25] displayed high similarity [**Fig. 2(C)**]. In addition, like pNI100 [8], the *sso* of pXT107 could form a structure of stem-loops (**Fig. 3**). These results indicated a typical RCR mechanism of pXT107.

E. coli-Nocardia shuttle plasmid pHAQ22 propagates in *Nocardia* but not in *Streptomyces*

The *Pst*I fragment of pXT107, containing the intact *rep* gene (**Fig. 1**), was cloned into *E. coli* plasmid pQC156, which contained actinomycetes selection markers *tsr* and *melC* [26], to yield pHAQ22. Introduced by transformation into plasmid-free hosts including *N. corallina* 4.1037 and *S. lividans* ZX7, thiostrepton-resistant transformants were obtained in strains *N. corallina* 4.1037 with a transformation efficiency of 3×10^2 per microgram plasmid DNA, but not in *S. lividans* ZX7 (plasmid pIJ702 as positive control). Another constructed plasmid pHAQ20, which cloned the *Bam*HI fragment of pXT107 (disrupting the *rep*) into pQC156, could not propagate in 4.1037 or ZX7. These results indicated the essential function of the *rep* gene of pXT107 for replication.

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