



STUDY ON THE AUTONOMOUSLY REPLICATING PLASMID pLY601 from *AMYCOLATOPSIS ORIENTALIS* HCCB10007

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Introduction. The genus *Amycolatopsis* belongs to the *Nocardioform Actinomycetes* which are producers of medically important antibiotics, such as vancomycin and rifamycin. To date, only one autonomously replicating plasmid pA387 was found and developed to be a conjugative shuttle vector(1). The small dissociated plasmid pLY601 (Fig.1) was found in an industrial vancomycin producing strain, *A. orientalis* HCCB10007. To facilitate the genetic manipulation of industrially important *Amycolatopsis* species, development of new conjugative shuttle vector is necessary based on pLY601. Here we report the complete DNA sequence of plasmid pLY601. Sequence analysis of pLY601 was conducted to determine the genetic organization of this indigenous plasmid. We also constructed an *E.coli-Amycolatopsis* shuttle vector, the first such conjugative vector based on pLY601 replicon for use in *Amycolatopsis* sp.

Methods. Plasmid pLY601 from HCCB10007 was isolated and digested with restriction enzymes or amplified by PCR to get the aimed fragments. The fragments were individually cloned into the vector pLYZL102. The resulting plasmids were transferred into *Amycolatopsis* sp. according to the protocol(2). Copy number was determined as reference(3).

Results. Sequence analysis revealed that pLY601 is 34,219 bp in length and totally encodes 50 genes (CP003411). However, 43 of genes are functionally unknown. Plasmid pLY601 did not exhibit any significant DNA sequence homology to any other characterized plasmid.

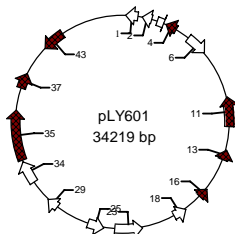


Fig.1 Map of plasmid pLY601. Parts of genes were showed in the map, shaded arrow means that the ORF can be analyzed.

After several rounds of validating, the replication region of plasmid pLY601 was focused to 2.8kb DNA fragment in bifunctional vector pLYZW7-3. Two complete ORFs (ORFs 23, 24) could be identified in this region, which did not exhibit any similarity to known replication protein available in databases.

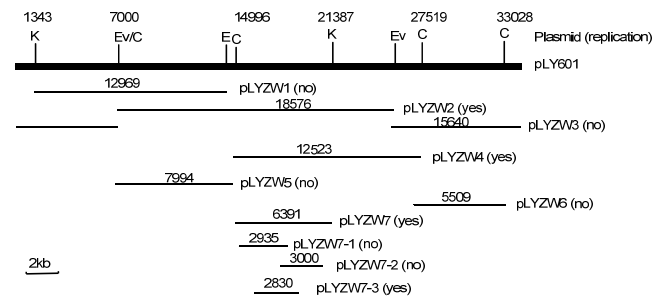


Fig.2 Identification of the minimal replication region. (K: *Kpn*I, Ev: *Eco*RV, E: *Eco*RI, C: *Cl*aI; plasmid pLYZW1, 2, 3, 4, 5, 6, 7, 7-1, 7-2, 7-3 were constructed as methods)

The copy number of vector pLYZW7-3 was about 15 in *A.orientalis*. The plasmid pLYZW7-3 is stably maintained in *A.orientalis* ATCC43490. To validate whether the vector was applicable to a wide range of *Amycolatopsis* species, the EGFP gene was cloned to the vector with *ermE* promoter. The resulted plasmid was transferred to *A.mediterranei* U-32, green fluorescent was visualized under fluorescence microscopy (Fig.3).

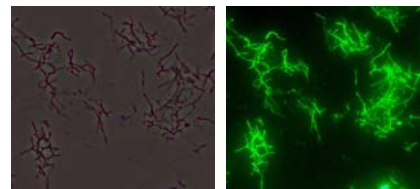


Fig.3 Analysis of EGFP synthesis by fluorescence microscopy(4). Magnification(40×), fluorescence images are on the right.

Conclusions. pLY601 was the second autonomously replicating plasmid found in *Amycolatopsis*. The bifunctional vector pLYZW7-3 with a broad repertoire could be applied in industrial *Amycolatopsis* for study in depth.

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References.

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