Sequence Analysis of Two Cryptic Plasmids from *Bifidobacterium longum* DJO10A and Construction of a Shuttle Cloning Vector

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Abstract

There are two major replication mechanisms for circular plasmid, namely, theta and rolling-circle replications (RCR). The most difference is that RCR plasmids generate the ssDNA intermediate and invariably contain double-strand origin (DSO), replication protein gene (*rep*), and single-strand origin (SSO) (1). The two plasmids, $p_{DOJH10L}$ and $p_{DOJH10S}$ isolated from *Bifidobacterium longum* DJO10A, were analyzed and found to consist of two circular DNA molecules of 10,073 and 3,661 bp, respectively. The $p_{DOJH10L}$, being a cointegrate plasmid, exhibited high sequence identity to two other RCR-type plasmids, $p_{NAC2}$ (98%) and $p_{KJ50}$ (96%). Interestingly, although the RCR regions of both the $p_{NAC2}$- and $p_{KJ50}$-like plasmids were disrupted during plasmid recombination, the $p_{DOJH10L}$ acquired a new functional replicon consisting of a fused *rep* gene and a DSO region. Further S1 nuclease analysis and southern hybridization to detect ssDNA intermediate were able to actually confirm $p_{DOJH10L}$ as a RCR-type plasmid (3). To the $p_{DOJH10S}$, sequence with high similarity to the theta Rep protein from *Rhodococcus rhodochrous* plasmid was identified. An *E. coli-B. longum* shuttle vector was then constructed utilizing $p_{DOJH10S}$ as basis, along with the *E. coli* ori, a *lacZ* gene, a MCS and a chloramphenicol resistance gene. This shuttle vector was successfully transformed into *E. coli* and *B. longum*, but failed in other lactic acid bacteria, indicating it is not a broad host vector. Its capability of residing in *B. longum* without antibiotic pressure for 92 generations was consistent with the general stability of Theta replication plasmids. This is the first cloning vector utilizing theta-type replicon in *Bifidobacterium* and should be useful for stably cloning foreign genes into *B. longum*.

Reference

