F328

Curing Of a Cryptic Plasmid From *B. lactofermentum*

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Brevibacterium lactofermentum KCTC 1844, a coryneform bacterium used for industrial production of various amino acids, has a cryptic plasmid pBL1. Several cloning vectors has been constructed using the plasmid pBL1, but they are not available for genetic manipulation of *B. lactofermentum* because of the incompatibility between the pBL1 and its derivatives. To solve the plasmid incompatibility, we tried to eliminate the plasmid pBL1 from *B. lactofermentum*. Because plasmid pBL1 has no known functions, it is not easy to detect the plasmid-free strain. It is, therefore, required to introduce the detectable phenotype into pBL1 in *B. lactofermentum* cells. The pBL1 was partially digested with *Sau*3AI, and followed by subcloning into the suicide vector containing a kanamycin resistance gene. The km^r transconjugants of *B. lactofermentum* were obtained by conjugal transfer of the recombinant plasmids from *E. coli* into *B. lactofermentum*. The *B. lactofermentum* transconjugants were analyzed to contain the same plasmid descriminated from pBL1 and the original recombinant plasmid, suggesting that the homologous recombination between pBL1 and the original recombinant plasmid was occurred *in vivo*. The km^s *B. lactofermentum* cells were efficiently obtained by growing the transconjugant in complex medium without kanamycin, and were identified to carry no plasmid. It was found that the plasmid was dramatically lost in the transconjugant at the elevated temperature. The resultant plasmid-free strain would be a useful host for pBL1-derived cloning vectors.