**BACILLUS STEAROTHERMOPHILUS PLASMID pSTK1 REPLICON IS FUNCTIONAL IN ESCHERICHIA COLI**

Issay Narumi¹, Noriyuki Nakayama², Shinya Nakamoto², and Hiroshi Kihara¹

¹Physics Laboratory, Department of Liberal Arts, Kansai Medical University, 18–89 Uyamahigashi, Hirakata, Osaka 573, Japan.
²NEC Corporation, 4–1–1 Miyazaki, Miyamae-ku, Kawasaki, Kanagawa 216, Japan.

**SUMMARY**

A kanamycin-resistant plasmid possessing a thermostable replicon derived from *Bacillus stearothermophilus* cryptic plasmid pSTK1 was constructed. The plasmid could transform not only *B. stearothermophilus* and *Bacillus subtilis*, but also Gram-negative *Escherichia coli*. The behavior of the plasmid in the hosts was examined. The plasmid was stably maintained even at 67°C in *B. stearothermophilus* without selective pressure. During the plasmid replication, single-stranded DNA (ssDNA) intermediates were found in *E. coli*, while these were not found in *B. subtilis*.

**INTRODUCTION**

During the practical application of *Bacillus stearothermophilus* plasmid systems, vectors that stably replicate in the host at elevated temperatures are desirable. In a previous report, we found a small cryptic plasmid pSTK1 from *B. stearothermophilus* strain TK015, and showed that the plasmid was stably maintained in *B. stearothermophilus* strain K1041 (Narumi et al., 1992) up to 70°C without changing its copy number (Narumi et al., 1993). Using the plasmid and *Escherichia coli* vector pUC19, a 5.7-kbp shuttle vector pSTE33 possessing a thermostable kanamycin-resistance gene was constructed. The thermostable nature of pSTK1 was inherited by pSTE33. This shuttle vector was stably maintained even at 67°C in strain K1041 without selective pressure, and its copy number remained almost constant at all growth temperatures of its host (Narumi et al., 1993).

The sequence analysis of pSTK1 reveals that open reading frame 1 (ORF1) of this plasmid has a helix–turn–helix motif typical of many DNA–binding proteins (Nakayama et al., 1993), and exhibits a significant degree of similarity with the Cop proteins (formerly RepA proteins) in a lactococcal plasmid pFX2 (Xu et al., 1991) and a streptococcal plasmid pLS1 (del Solar et al., 1989). Cop protein is considered to control replication of the plus-strand of some rolling circle replication (RCR) plasmids as a transcriptional repressor (del Solar et al., 1993). Also identified by sequencing of pSTK1 is a region that can form an extensive hairpin structure, which would show a high degree of similarity with *palA*, an origin for minus–strand elongation in RCR plasmids (Novick, 1989). The presence of *palA*-like region suggests that the pSTK1 replicon should act with RCR mechanism. In pSTK1, however, no significant homology was detected with any plus-strand origins or replication initiation proteins (Rep proteins) found in other RCR plasmids (Nakayama et al., 1993).

This paper describes 1) the pSTK1 replicon from Gram-positive plasmid functions in Gram-negative *E. coli*, and 2) the ssDNA replication intermediates were detected in *E. coli* harboring a pSTK1 derivative.

---

*Present address: Biotechnology Laboratory, Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, 1233 Watanuki, Takasaki, Gunma 370–12, Japan.
*To whom correspondence should be addressed.
MATERIALS AND METHODS

Bacterial strains and plasmids: *B. stearothermophilus* K1041, *B. subtilis* RM125, and *E. coli* JM109 were used as hosts, and transformed as described (Narumi et al., 1993). A shuttle vector pSTE33 (Narumi et al., 1993) was digested with EcoRI, HindIII, and also with AlwNI to shorten a pUC-derived fragment containing the ori region and the ampicillin-resistance gene. A 3.0-kbp EcoRI–HindIII fragment containing whole pSTK1-derived region and a thermostable kanamycin-resistance gene was blunted with T4 DNA polymerase and self-ligated with T4 DNA ligase (Takara Shuzo Co. Ltd.). The resulting plasmid from strain K1041 transformants was designated pSTK5. A schematic presentation of pSTK5 is shown in Fig. 1 with its parental plasmids.

![Plasmid structures of pSTK1 and its derivatives.](image)

**Fig. 1.** Plasmid structures of pSTK1 and its derivatives.

Plasmids are represented to size. Open box indicates DNA sequence derived from pSTK1. Oblique box indicates the *psaA*-like region. Striped and blackened boxes indicate DNA sequences derived from pKM141 (Narumi et al., 1993) and pUC19 (Yanisch-Perron et al., 1985), respectively. Arrows represent the orientation of transcription. Abbreviations: N, NruI; B, BamHI; H, HindIII; E, EcoRI; ORF, open reading frame; pUC-ori, replication origin of pUC19; Amp r, ampicillin resistance determinant; Km r, kanamycin resistance determinant.

Preparation of total DNA lysates: Cells harboring plasmid pSTK5 were grown under selective and non-selective conditions at constant temperatures (at 37 and 42°C for *E. coli*; at 37 and 52°C for *B. subtilis*). Total DNA lysates were prepared from the cells as described (Narumi et al., 1993), and used for the plasmid copy number determination and the southern blot hybridization analysis. Plasmid copy number was determined as previously described (Narumi et al., 1993).

Southern blot hybridization: Plasmid pSTK5 purified from strain K1041 was linearized with BamHI, and labeled with digoxigenin–ddUTP (DIG) according to the manufacturer's instruction (Boehringer Mannheim). Agarose gel (1%) electrophoresis of the total DNA lysates and southern blotting to a nylon membrane were performed according to standard protocols (Sambrook et al., 1989). If necessary, the total DNA lysates were treated with 1 u of S1 nuclease (Takara Shuzo Co. Ltd.) for 20 min at 37°C before subjected to agarose gel electrophoresis. Prehybridization, hybridization, and washing step in a low salt concentration buffer were performed at 68°C. Signal detection was carried out with the colorimetric detection reagents in a DIG-Detection Kit (Boehringer Mannheim).

Plasmid segregational stability: *B. stearothermophilus* K1041 harboring plasmid pSTK5 was cultivated at 47, 57, and 67°C until 21 generations, then segregational stability was assayed as previously described (Narumi et al., 1993). The stabilities of pSTK5 in *B. subtilis* (37 and 52°C) and in *E. coli* (37 and 42°C) were also examined in the same way.

Nucleotide sequence accession number: The sequences of plasmids pSTK5 and pSTE33 have been assigned in the DDBJ, EMBL, and GenBank accession number D29978 and D29979, respectively.

RESULTS

Transformation with plasmid pSTK5

Plasmid pSTK5 could transform *B. stearothermophilus* K1041 to kanamycin–resistance at a frequency of $3.2 \times 10^6$ c.f.u. per µg DNA by electroporation, and could transform *B. subtilis* RM125 by protoplast transformation similar to its parental plasmid pSTE33 (Narumi et al., 1993). Surprisingly, pSTK5 could transform *E. coli* JM109 in spite of a lack of the pUC19 replicon. The transformation efficiency of pSTK5 for *E. coli* by electroporation was $1.2 \times 10^4$ c.f.u. per µg DNA.