COMPLETE NUCLEOTIDE SEQUENCE OF pSTK1, A CRYPTIC PLASMID FROM BACILLUS STEAROTHERMOPHILUS TK015

Noriyuki Nakayama1*, Issay Narumi2*, Shinya Nakamoto1, and Hiroshi Kihara2*

1NEC Corporation, 4-1-1 Miyazaki, Miyamae-ku, Kawasaki, Kanagawa 216, Japan.
2Jichi Medical School, School of Nursing, 3311-159 Yakushiji, Minamikawachi, Tochigi 329-04, Japan.

SUMMARY
The complete nucleotide sequence of pSTK1, a cryptic plasmid isolated from B. stearothermophilus TK015, has been determined. pSTK1 has been shown to be 1883 bp in length and contain three open reading frames (ORFs), one of which has a helix-turn-helix motif typical of DNA-binding proteins. Also identified was a region that can form an extensive secondary structure, which would show a high degree of similarity to pala, an origin for minus strand elongation in rolling circle replication.

INTRODUCTION
The 1.9-kbp cryptic plasmid pSTK1 was originally isolated from B. stearothermophilus TK015, and it was stably maintained in its host up to 70 °C without changing its copy number. The thermostable nature of pSTK1 was inherited by plasmid pSTE33, which is a shuttle vector constructed from pSTK1, plasmid pUC19, and a thermostable kanamycin-resistance marker (Narumi et al., 1993). The thermostability and host range of pSTE33 make it useful for cloning experiments using B. stearothermophilus as a host.

The part of pSTE33 nucleotide sequence corresponding to pUC19 (Yanisch-Perron et al., 1985) and the thermostable kanamycin nucleotidyltransferase gene (Liao et al., 1986) is known. This paper describes 1) the complete nucleotide sequence of pSTK1, which is the key to the thermostable replication of pSTE33 in B. stearothermophilus, and 2) homologies of pSTK1 with other plasmids.

MATERIALS AND METHODS
Bacterial strains and plasmid: Escherichia coli JM109 and plasmid pUC19 were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). B. stearothermophilus TK015 was isolated from soil by the authors (Narumi et al., 1993).
DNA manipulations: Plasmid DNA was extracted according to standard protocols (Sambrook et al., 1989) and purified with a QIAGEN column (Diagen GmbH, Hilden, Germany). Plasmid pSTK1 was purified by agarose gel electrophoresis and electroelution, because B. stearothermophilus TK015 was shown to harbor three different plasmids (Narumi et al., 1993). E. coli JM109 cells were transformed by electroporation (Dower et al., 1988). Restriction digestions, alkaline phosphatase treatments, blunting treatments, and ligations were carried out as recommended by the supplier (Takara Shuzo Co., Ltd.).
DNA sequence determination: Plasmid pSTK1 was linearized with NspI, which cuts it a unique site, and inserted into the Sphi site of pUC19 to construct plasmid pSTE90. Deletion derivatives from pSTE90 were constructed using a Deletion Kit (Takara Shuzo Co., Ltd.). Plasmid pSTE90 and its deletion derivatives were proliferated in E. coli JM109 and used for sequencing. Plasmid pSTK1 was also sequenced in the area about the NspI site. Sequencing was performed on both strands of DNA by the dideoxynucleotide chain termination method, using a double-stranded DNA template, an AmpliTaq

*Present address: Physics Laboratory, Department of Liberal Arts, Kansai Medical University, 18-89 Uyamahigashi, Hirakata, Osaka 573, Japan.
RESULTS

Nucleotide sequence of pSTK1

Plasmid pSTK1 was 1883 bp in length (Fig. 1). The numbering of the sequence

Fig. 1. Nucleotide sequence of pSTK1 and amino acid sequences of ORFs. The putative promoter sequences and ribosomal binding sites (RBS) are underlined. Palindromic sequences are indicated by arrows; dots show positions of nonpalindromic nucleotides. The region that assumes a pAILike secondary structure is also indicated.