The **Bacillus subtilis dnaC** gene encodes a protein homologous to the DnaB helicase of *Escherichia coli*

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Within the region of the *Bacillus subtilis* chromosome assigned to us in the genome sequencing project, we found a gene, the product of which is similar to the DnaB protein (replicative DNA helicase) of *Escherichia coli*. Three *B. subtilis* dna gene mutations, dnaC30 and ts56 causing defects in elongation and ts199 causing a defect in the initiation of replication, were mapped in the gene by transformation and DNA sequencing. Both dnaC30 and ts56 have been located near the amino-terminal end of the *B. subtilis* DnaC protein. In contrast, ts199 has been located near the carboxy-terminal of the protein. Our results indicate that the *B. subtilis* dnaC gene encodes a counterpart of the *E. coli* dnaB helicase.

**Keywords**: *Bacillus subtilis*, genome sequencing, dnaC, DNA helicase

The DnaB protein of *Escherichia coli* acts as a central replicative DNA helicase and therefore is essential for initiation and elongation in chromosome replication. In *Bacillus subtilis*, its counterpart has not yet been identified. We have recently found a gene (ORF454) near the *purA* gene in the 180 kb region assigned to us as part of the *B. subtilis* genome sequencing project, the deduced amino acid sequence of which shows 42% identity to *E. coli* DnaB helicase (Ogasawara et al., 1994). The organization of ORFs around this gene is shown in Fig. 1. The dnaC mutation and two other groups of dna-ts mutations (tsA and tsB) have already been mapped near *purA* on the *B. subtilis* genetic map (Karamata & Gross, 1970; Bazill & Retief, 1969; Andersen & Ganesan, 1975; Hara & Yoshikawa, 1973). The dnaC and tsA mutations were defective in elongation, whereas tsB mutations were defective in initiation. Although dnaC and tsA,B were mapped at different but close loci on the *B. subtilis* genetic map (Anagnostopoulos et al., 1993), the relationship between dnaC and tsA,B was not examined (Yoshikawa & Wake, 1993). To find which of the dna mutations were located on ORF454, three dna mutant strains [ts56 and ts199 (representatives for the tsA and tsB group, respectively) and dnaC30] were used as recipients for transformation. Donor DNA was derived from four λ clones (D3, D25, PL and PL2–3). As shown in Fig. 1, the DNA of three clones (D3, D25 and PL) did not revert the temperature-sensitive phenotype of any mutant, whereas PL2–3 DNA transformed all mutants with significantly high efficiency. These results indicate that the three markers are located close to each other and in the vicinity of ORF454. To map these mutation sites more precisely, the DNA of three other clones (PL2, YS1 and YS2) was assayed for its ability to complement them. Fig. 1 shows that the DNA of all three clones transformed dnaC30 and ts56 mutant cells, indicating that both mutations are located on a common fragment (from an EcoRI site in ORF454 to a HindIII site in ORF66). In contrast, when a ts199 strain was used as recipient, only YS1 DNA had transforming activity, suggesting that the ts199 mutation is located in the 3′ region of ORF454. These results suggest that all three dna mutations are located in ORF454.

The *B. subtilis* purA gene was cloned based upon its sequence similarity to the *E. coli* counterpart (Mantsala & Zalkin, 1992). In this study we have confirmed that the *B. subtilis* purA16 mutation is situated in a sequence containing this gene (Fig. 1).

As both dnaC30 and ts56 were shown to be present in an EcoRI–HindIII DNA fragment of 880 bp, the nucleotide sequence of the region in both mutant chromosomes was determined to identify these mutation sites. Furthermore, the nucleotide sequence of the 2.15 kb fragment (corresponding to the insert in clone YS1) of the ts199 chromosome was determined to identify the ts199.
mutation site. In each case, a single base pair change was found in comparison with the wild-type sequence and the change caused a missense mutation in the coding region of ORF454. Thus, aspartic acid (residue 42) was replaced by glycine (codon change from GAT to GGT) in ts56, threonine (residue 67) was replaced by isoleucine (ACA to ATA) in ORF454, and alanine (residue 350) was replaced by valine (GCC to GTG) in ts199. These results demonstrate that ORF454, encoding a protein similar to B. subtilis DnaC helicase, is identical to the dnaC gene. Furthermore, both ts56 and ts199 are mutant alleles of dnaC. Therefore, hereafter, we call ts56 and ts199, dnaC56 and dnaC199, respectively. It was also shown that two types of mutants defective in elongation (dnaC30 and dnaC56) and in initiation (dnaC199) can be isolated, both affected by the B. subtilis dnaC gene. Similarly, two types of dna mutant were reported for the dnaB gene of E. coli (Wechsler & Gross, 1971; Zyskind & Smith, 1977).

Nakayama et al. (1984a) determined that the E. coli DnaB protein could be divided by trypsin digestion into two relatively stable domains, a 12 kDa amino-terminal (fragment III) and a 33 kDa carboxy-terminal domain (fragment II). About 45 amino acids between fragments II and III serve as a hinge region. In addition, 14 amino acids of the amino-terminal end are also lost during the cleavage. In Fig. 2, the amino acid sequence of B. subtilis DnaC helicase is compared with three other helicases, E. coli and Salmonella typhimurium dnaB helicases and B. subtilis phage SPP1 replicative DNA helicase. In previous studies, the carboxy-terminal portions of bacterial and bacteriophage primase-associated helicases were found to be conserved (Ilyina et al., 1992; Pedre et al., 1994). The DnaC protein of B. subtilis also showed significant similarity in its carboxy-terminal half with the three other helicases, in that the A and B motifs involved in purine NTP-binding and hydrolysis (Walker et al., 1982) were conserved (Fig. 2). Significant similarity was also found in the remaining portion among bacterial helicases, except that the amino-terminal 14 amino acids were missing in B. subtilis (Fig. 2).

The dnaC199 mutation identified in this study was found to be located near the carboxy-terminal. Since the dnaC199 mutant is defective in the initiation of replication, it is unlikely that ATP-binding and/or ATPase activity are also affected in this mutant, since ATP hydrolysis was shown to be essential for the helicase action of E. coli DnaB (LeBowitz & McMacken, 1986) and the deficiency would create mutants defective in elongation, not initiation. The carboxy-terminal domain of B. subtilis DnaC seems to be participating in protein–protein interaction, analogous to the situation in E. coli where this domain in E. coli DnaB is able to form a hexamer (Nakayama et al., 1984a), and probably interact with the λ P protein (Chang et al., 1991). The mutant DnaC199 protein might not be able to interact with a carrier protein like the E. coli DnaC protein, although the amino-terminal domain of DnaB is essential for this interaction in E. coli (Nakayama et al., 1984a).
In contrast to the high similarity of the carboxy-terminal domain in the four replicative DNA helicases, the amino-terminal domain showed less similarity (Fig. 2). The dnaC56 and dnaC30 mutations, defective in elongation, were located in this domain. The amino acids in the wild-type sequence corresponding to those changed in the mutants were conserved in the bacterial helicases. Nakayama et al. (1984a) suggested that the amino-terminal domain of E. coli DnaB is essential for interaction with primase and DnaC. Therefore, an interaction of B. subtilis DnaC with primase might be impaired in both dnaC56 and dnaC30 mutants, resulting in a deficiency in elongation.

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