Plasmid Instability in Bacillus subtilis during Sporulation

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Received August 10, 1992

The plasmid pC194 has been widely used in studies of plasmid transformation and replication in Bacillus subtilis. It is stably maintained in the bacterium, and a region that encodes a gene that is required for the stable inheritance of the plasmid is well studied. Although intensive work on plasmid replication and segregational stability during vegetative growth in B. subtilis has been done, very few studies have been done in these areas for the period of sporulation. This paper describes the stability of plasmid pC194 during sporulation.

B. subtilis MI113 arg-15 trpC2 hisM hsrM was used as a host strain of the plasmid, which is a derivative of the standard strain Marburg 168. Competent cells of the bacterium were prepared by the method of Anagnostopoulos and Spizizen with the slight modification of adding required amino acids (50 and 5 μg/ml for the first and second growth media, respectively). Transformants were selected on L agar plates (10 g of Polypepton, 5 g of yeast extract, 5 g of NaCl, and 20 g of agar per liter [pH 7.0]) containing 5 μg/ml of chloramphenicol (Cm). The spores were counted as the number of colonies appearing on the plates with appropriate dilution after heat treatment of the culture broth for 15 min at 80°C. All the vegetative cells were confirmed to have been killed after a 5-min heat treatment at 60°C, while no changes in the numbers of spores were observed, even after a 20-min treatment at 80°C. Thus, the number of spores in the culture broth, which contained spores and vegetative cells, was counted selectively by the specific killing of the vegetative cells with a 15-min heat treatment of the broth at 80°C. The number of spores and the total number of viable cells, consisting of spores and vegetative cells, are both expressed as colony forming units (CFU).

The stability of the plasmid in B. subtilis MI113 during vegetative growth was examined as follows. A plasmid-carrying transformant was cultured in the presence of chloramphenicol at 37°C to the logarithmic growth phase, and 1% of this culture was inoculated into fresh LB medium with the antibiotic. After four successive cultivations, no spores were detected in the culture liquid. The vegetative cells were grown for about 21 generations under non-selective conditions, and the culture was spread on L-agar plates after dilution. The cell generations were counted by the number of the cells at the initial and final points. One hundred colonies selected randomly were transferred by replica plating onto assay plates with and without chloramphenicol. The fraction of plasmid-carrying cells in the total population was 99%. This result indicates that plasmid pC194 was maintained stably at 37°C in this host strain during vegetative growth, as previously reported.

The stability of the plasmid during sporulation was examined as follows. Spores were formed in Schaeffer-sporulation medium (5 g of Difco nutrient broth, 0.25 g of MgSO4·7H2O, 1.0 g of KCl, 0.001 mM FeSO4, 1 mM Ca(NO3)2, and 0.01 mM MnCl2 per liter) after 12 h of cultivation of the transformant at 37°C with and without chloramphenicol. Plasmid stability in spores is expressed as the fraction of the plasmid carrier among colonies that appeared after heat treatment (80°C, 15 min). The presence of the plasmid in the antibiotic-resistant colonies and its disappearance in the antibiotic-sensitive colonies were confirmed by alkaline extraction of ten randomly-selected colonies followed by agarose gel analysis. No changes in molecular weight of the plasmid by deletion or insertion were observed. The plasmid stabilities were 64% and 56% from the spores made under non-selective and selective conditions, respectively. This means that high-frequency curing of the plasmid occurred irrespective of the presence of chloramphenicol when the spores were formed.

As significant curing was observed in the spores, changes in the numbers of viable cells and spores and in the plasmid stabilities were measured periodically in L medium without chloramphenicol for 4 days. The cells were cultivated for about 12 generations. As shown in Fig. 1, plasmid stabilities in spores were consistently about 50 to 60%, while the total cell stability declined from an initial 100% to 50% according to the sporulation.

Fig. 1. Plasmid Stability during Cell Growth and Sporulation in L Medium without Chloramphenicol.

(A) Changes in total cell number (○) and in the number of spores (●) during cultivation in L medium at 37°C. After successive cultivation of vegetative cells, no spores were detected at the start of this experiment.

(B) Plasmid stabilities expressed as the plasmid-holding capacity of total cells (○) and of spores (●).

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septum partitions the developing cell into two unequal compartments, the forespore and the mother-cell, each of which carries a chromosome from the last round of vegetative DNA replication. After this initial stage, the septum migrates around the forespore which is enclosed within the mother-cell; the plasmid instability observed in this work is rationally suggested to have occurred in this initial stage. Some kind of exclusive function against plasmid DNA might exist during the septum formation in the developing cell. An alternative possibility is the non-homogeneous distribution of plasmids in the cytosol. Genes required for stable maintenance of plasmids in bacterial cells have been identified on Escherichia coli plasmids. Also, in B. subtilis the par function is reported to reside on plasmids pLS11, pTA1060, and pBAA1, but is not known to work or not to stabilize the plasmid at the stage of sporulation.

The elucidation of plasmid stability during sporulation, as well as during vegetative growth, will be important in field application of genetically manipulated microorganisms by furthering our understanding of the partitioning of plasmids in spore-forming bacteria.

Acknowledgment. We are grateful to T. Tanaka of Tokai University, for his generous gifts of the bacterial strain and plasmid.

References