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GENOME ANNOUNCEMENT

Complete Sequence of the First Chimera Genome Constructed by Cloning the Whole Genome of *Synechocystis* Strain PCC6803 into the *Bacillus subtilis* 168 Genome

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Genome synthesis of existing or designed genomes is made feasible by the first successful cloning of a cyanobacterium, *Synechocystis* PCC6803, in Gram-positive, endospore-forming *Bacillus subtilis*. Whole-genome sequence analysis of the isolate and parental *B. subtilis* strains provides clues for identifying single nucleotide polymorphisms (SNPs) in the 2 complete bacterial genomes in one cell.

A method to connect small DNA segments in *Bacillus subtilis*, originally termed megacloning, enabled the combination of a whole genome from *Synechocystis* PCC6803 with the *B. subtilis* genome (3). When the project started in 1997, the sequence of the whole bacterial genome to be used was the minimal requirement for such an approach. This prerequisite, combined with the fact that the strain is not harmful to humans, led us to choose the *Synechocystis* PCC6803 strain for the project. The chimeric genome strain BEST7613 (3) has raised a number of poorly argued issues (1). To the best of our knowledge, growth of *Synechocystis* in a suitable medium, a complete synthetic medium for photosynthesis with no carbon sources, has not been achieved yet; therefore, BEST7613 must always be cultivated in a *B. subtilis* growth medium. Given that the genes from the *Synechocystis* genome are properly expressed, conversion of the cellular gene regulatory network from that of *B. subtilis* to that of the other partner is expected. A number of factors and components that were provided by a switch in gene expression from *B. subtilis* to *Synechocystis* in response to changes in the culture medium remain to be examined. These include structures on the inside and outside of the cell membrane and cell wall and the metabolic state. To obtain clues, sequence information on BEST7613 and its precursor, the *B. subtilis* strain BEST7003, was also determined.

The genomes were sequenced in a multiplexed, paired-end, 100-bp manner on a Genome Analyzer II (Illumina, San Diego, CA) to generate 60- and 80-fold coverage. *De novo* assemblies were performed using Velvet software version 1.1.02 (6). For the BEST7003 strain, 53 scaffolds were aligned to the *B. subtilis* 168 genome using MUMmer (4). For the BEST7613 strain, 243 scaffolds were sorted according to strain constriction procedures (3). The genome sequences of strains BEST7003 and BEST7613 consisted of 4,043,042 bp and 7,585,470 bp, respectively. A total of 4,011 (BEST7003) and 7,270 (BEST7613) genes were determined using the Microbial Genome Annotation Pipeline (MiGAP) (http://www.migap.org/).

BEST7003 was constructed based on *B. subtilis* RM125 (3), which contains 2 rearrangements in its genome: the integration of the *Bacillus amyloliquefaciens* genome around the *purB* gene and the dislodgment of the SPβ phage region (2). Our sequence analysis revealed both rearrangements in the BEST7003 genome. We found 2 large deletions in the BEST7613 genome. The skin element was dislodged, leading to the reconstitution of the *sigK* gene. This phenomenon is caused by the sporulation process (5). We also found a deletion of the *Synechocystis* genome region (bp 1617467 to 1624268) containing sll1411 to sll2111. Since the region contains repetitive sequences, which includes 4 transposase genes, it may be reformed by homologous recombination. In addition, we found several single nucleotide polymorphisms (SNPs) in the *Synechocystis* genome region, although there were no mutations that disrupted photosynthesis. A detailed report of these genomes will be included in future publications.

Nucleotide sequence accession numbers. The genome sequences for these strains have been deposited at DDBJ/EMBL/GenBank under accession numbers AP012496 (BEST7003) and AP012495 (BEST7613).

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