

Complete Genome Sequence of Highly Multidrug-Resistant *Pseudomonas aeruginosa* NCGM2.S1, a Representative Strain of a Cluster Endemic to Japan

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Received 2 October 2011/Accepted 3 October 2011

We report the completely annotated genome sequence of *Pseudomonas aeruginosa* NCGM2.S1, a representative strain of a cluster endemic to Japan with a high level of resistance to carbapenem (MIC \geq 128 μ g/ml), amikacin (MIC \geq 128 μ g/ml), and fluoroquinolone (MIC \geq 128 μ g/ml).

The emergence of multidrug-resistant (MDR) *P. aeruginosa* strains is a serious problem in Japan (2, 6, 7). The MDR *P. aeruginosa* strain NCGM2.S1 caused an outbreak of urinary tract infection at a hospital in Miyagi Prefecture, northern Japan (6). NCGM2.S1 harbors a metallo- β -lactamase gene, *blaIMP-1*, and an aminoglycoside 6'-*N*-acetyltransferase gene, *aac(6')-Iae*, in the class I integron In113 (6). Epidemiological studies indicated that clonal expansion of NCGM2.S1 occurred in hospitals in this area (7) as well as other areas in Japan (4, 8). We developed kits to detect *aac(6')-Iae* and AAC(6')-Iae, which were used to survey MDR *P. aeruginosa* strains (3, 7).

The genome of *P. aeruginosa* was sequenced using a Roche FLX Titanium genome sequencer. We obtained a total of 532,063 reads, covering a total of 6,697,230 bp, or 28.9-fold coverage. Sequences were assembled into a total of 270 contigs. Gaps were filled by Sanger sequencing of PCR products by brute force amplification of the regions between contigs. Primary CDS extraction and initial functional assignment were performed using the RAST automated annotation servers (1). The results were compared to verify the annotation and were corrected manually by *in silico* molecular cloning (In Silico Biology, Inc., Kanagawa, Japan). The *P. aeruginosa* NCGM2.S1 genome consists of a single circular chromosome of 6,764,661 bp, with an average GC content of 66.1%. The chromosome was shown to contain a total of 6,271 protein-coding genes, 77 tRNA genes, 1 tmRNA for all amino acids, and 4 *rrn* operons. In addition, the chromosome harbors 6 prophage-like elements.

Although *P. aeruginosa* NCGM2.S1 is a representative strain of an endemic cluster showing a high level of multidrug resistance in Japan, it does not have any plasmids. Instead, the chromosome was shown to harbor the class I integron In113 carrying *aac(6')-Iae* and *blaIMP-1*, which are responsible for

high levels of resistance to aminoglycosides and β -lactams, respectively. Of note, In113 is inserted into the middle of *oprD*, resulting in complete disruption of the gene. OprD is responsible for sensitivity to imipenem, and its reduced expression increases the level of resistance (5). Analysis of the complete NCGM2.S1 genome sequence strongly suggested that *P. aeruginosa* acquires drug resistance not only by obtaining drug resistance genes but also by disrupting the genes involved in drug sensitivity.

Nucleotide sequence accession number. The nucleotide sequence of the chromosome of *P. aeruginosa* NCGM2.S1 has been deposited in the DNA Database of Japan under accession no. AP012280.

We thank Y. Sakurai for excellent work in the genome analysis.

This study was supported by a Grant for International Health Research (GIHR) (21A-105) from the Ministry of Health, Labor, and Welfare (MHLW). T.K. was supported by a grant (H21-Shinko-ippan-008) from MHLW. T.M.-A. was supported by a GIHR (23A-301) from MHLW.

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