

Genome Sequence of the Nonpathogenic *Listeria monocytogenes* Serovar 4a Strain M7[∇]

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This report presents the complete and annotated genome sequence of the naturally nonpathogenic *Listeria monocytogenes* serovar 4a strain M7, isolated from cow's milk in Zhejiang province, China.

Listeria monocytogenes encompasses a diversity of strains with various virulence and pathogenicity levels (10, 19). Serovars 4b, 1/2a, and 1/2b account for the majority of human listeriosis cases, whereas other serovars, particularly serovar 4a, are rarely associated with listeriosis and are seldom isolated from foods (3, 18). To obtain a better understanding of the molecular mechanisms of *L. monocytogenes* pathogenicity, the genome sequence of the naturally nonpathogenic serovar 4a strain M7, isolated from cow's milk in Zhejiang province, China (2), was analyzed.

The complete genome sequence of M7 was determined with a Solexa Genome Analyzer II platform (100-bp paired-end sequencing library with an insertion size of 500 bp) at Genergy Biotechnology (Shanghai, China). Assembly was performed using SOAPdenovo. Gaps were closed by primer walking and sequencing of PCR products. Coding sequences (CDSs) were predicted using GenDB v2.2 (11) and Glimmer 3.02 (5) and compared with a nonredundant protein database to obtain descriptions. Reference genome sequences were obtained from GenBank for five pathogenic *L. monocytogenes* strains including serovar 1/2a strains EGD-e (NC_003210) (8), 08-5578 (NC_013766), and 08-5923 (NC_013768) (7) and 4b strains F2365 (NC_002973) (12) and CLIP80459 (NC_012488), as well as *Listeria innocua* strain CLIP11262 (NC_003212) (8), *Listeria welshimeri* strain SLCC5334 (NC_008555) (9), and *Listeria seeligeri* strain SLCC3954 (NC_013891) (17).

The genome of M7 consists of a single circular chromosome of 2,976,163 bp, with a GC content of 38.2%. There are 2,977 CDSs that represent 89.2% of the genome, with an average length of 297 amino acids, six 16S-5S-23S rRNA operons, and 67 tRNA genes. M7 harbors no plasmid and carries three prophages bearing 53, 78, and 66 genes, respectively. The genome of M7 is highly similar to that of HCC23, a serovar 4a strain isolated from catfish in the United States, which contains 2,976,212 nucleotides (NC_011660) (16). Genomic differences were observed on

68 loci (12 deletions, 45 insertions, and 11 substitutions) between HCC23 and M7.

Comparative analysis of six *L. monocytogenes* genomes (F2365, CLIP80459, EGD-e, 08-5578, 08-5923, and M7) revealed that the *prfA* locus, internalin genes *inlA* and *inlB*, and sugar phosphate permease gene *hpt* were conserved in *L. monocytogenes*. Notably, one mutation (G145S) in the positive transcriptional regulator (PrfA) that results in its constitutive activation (6) was identified in M7 but absent in other strains, including HCC23. Sixty-four genes that were conserved in pathogenic strains were absent in M7 (prophage genes excepted). At least three internalins (InlC, InlH, and InlJ) that contribute to *Listeria* pathogenicity were absent in M7 (13, 14, 15). On the other hand, 151 genes were identified as specific to M7 but absent in pathogenic strains (except the prophage genes). Twenty of these genes were also present in *L. innocua*, a nonpathogenic species closely related to *L. monocytogenes* (4).

A total of 2,168 genes constitute the core genome of *L. monocytogenes*, *L. innocua*, *L. welshimeri*, and *L. seeligeri*. The phylogenetic tree based on these genes confirms that M7 constitutes an evolutionary intermediate between *L. monocytogenes* and *L. innocua* (1).

Nucleotide sequence accession number. The complete genome has been deposited in GenBank under accession number CP002816.

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