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Complete Genome Sequence of Newcastle Disease Virus Mesogenic Vaccine Strain R2B from India

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Mesogenic vaccine strains of Newcastle disease virus (NDV) are widely used in many countries of Asia and Africa to control the Newcastle disease of poultry. In India, the mesogenic strain R2B was introduced in 1945; it protects adult chickens that have been preimmunized with a lentogenic vaccine virus and provides long-lasting immunity. In this article, we report the complete genome sequence of the hitherto unsequenced Indian vaccine virus strain R2B. The viral genome is 15,186 nucleotides in length and contains the polybasic amino acid motif in the fusion protein cleavage site, indicating that this vaccine strain has evolved from a virulent virus. Phylogenetic analysis of this mesogenic vaccine virus classified it with the viruses belonging to genotype III of the class cluster II of NDV.

Newcastle disease virus (NDV) is a highly contagious and fatal disease-causing virus that affects most species of birds worldwide (5, 6). The genome of the virus, which is approximately 15 kb in size, is of negative polarity and nonsegmented in nature. The virus encodes six main structural proteins, namely, nucleocapsid (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin (HN), and large (L) proteins. Both live attenuated and inactivated vaccines have been developed to control NDV infections. The vaccine strain R2B used in the Indian subcontinent has given excellent results in older birds (>6 to 8 weeks old) with long-lasting immunity but has proven to be severely pathogenic for baby chicks. The virus strain had originated by passaging three Indian field isolates in embryonated chicken eggs, with one of the lines showing signs of attenuation after 19 passages (3). The subsequent work was taken over in Mukteswar, India, and the attenuation was carried further, over 90 passages, and was successfully tested in vaccine trials (2).

The plaque-purified R2B strain seed virus, available from the viral repository of Indian Veterinary Research Institute, was propagated in 11-day-old specific-pathogen-free embryonated chicken eggs via the allantoic route. Viral genomic RNA was isolated from the infected allantoic fluid using TRIzol LS reagent (Invitrogen, CA). The 3′ leader and 5′ trailer sequences of the virus were determined using 3′ rapid amplification of cDNA ends (3′ RACE) and 5′ RACE, respectively (4). The complete genome of strain R2B was determined by an overlapping reverse transcription-PCR strategy (ABI 3730 DNA analyzer; Applied Biosystems). The length of the genome of NDV strain R2B is 15,186 nucleotides and is similar to the genome length of most of the members of the genus *Avulavirus*. The fusion protein cleavage site (FPCS) of NDV is a major virulence determinant marker and is suggested by the presence of multiple basic amino acids, followed by phenylalanine at position 117, which is indicative of a virulent virus (1). The FPCS of strain R2B is ^{111}GRRQKRF^{117}, suggesting that this vaccine strain is a derivative of a virulent virus.

The complete genome sequence of strain R2B was further analyzed phylogenetically together with 148 NDV genomes available in GenBank, using the maximum likelihood method with the MEGA5 software (7). The R2B strain aligned closely with the NDV vaccine strain Mukteswar (JF950509.1, EF201805.1) reported from China and two velogenic isolates from China (Newcastle disease virus isolate JS/7/05/Ch, FJ430159.1, and Newcastle disease virus isolate JS/9/05/Go, FJ430160.1) and was placed under genotype III of the class II cluster of NDV. Interestingly, at the nucleotide level, the polymerase gene, which is also associated with the virulence of the virus, showed 99% homology with a velogenic Egyptian NDV strain (NDV/Chicken/Egypt/1/2005, FJ939313.1). The genome sequence information of the R2B vaccine strain will open up new vistas in developing new multivalent vaccines by reverse genetic techniques.

Nucleotide sequence accession number. The complete genome sequence of mesogenic vaccine strain R2B from India has been deposited in GenBank under the accession number JX316216.

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