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Membrane and Envelope Gene Sequence Analysis and Characterization of an Indian Avian Infectious Bronchitis Virus Vaccine Strain

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Indian avian infectious bronchitis virus (IBV) vaccine strains isolated from live attenuated commercial IBV vaccine was characterised based on M and E gene sequence. RNA was extracted and RT-PCR technique was performed to amplify the M and E gene sequence encoding membrane and envelope protein of IBV. Recombinant plasmid named pTZ57R/T-M and pTZ57R/T-E was constructed via inserting the M and E gene into the TA cloning vector, pTZ57R/T respectively. The sequenced M and E gene and its deduced amino acid sequences were compared with the published sequences of reference strains. The M and E gene is of 678 bp and 330 bp in length encoding 225 amino acids and 109 amino acids with a predicted molecular weight of 25.5 kDa and 12.46 kDa respectively. The sequences of the M and E gene share 96.8-99.7% and 82.1-100% homologous identities at nucleotide level with published reference vaccine strain and field isolate strains from different regions and countries respectively. E gene showed significant variation in sequence identities. Phylogenetic tree based on these M and E sequences was generated, and the tree topology suggests that Indian IBV vaccine strain share common ancestor with routinely used reference vaccine strain sequences.

Key words: Infectious bronchitis virus (IBV), M gene, E gene, Phylogeny, Vaccine.

India's poultry industry which represents a major success story with 8 percent rise in poultry production per annum[1]. Various poultry diseases like Newcastle disease (NDV), Infectious Bursal Disease (IBD) and Infectious bronchitis (AIB) causes great losses to poultry industry. Avian infectious bronchitis is a highly contagious, acute and ubiquitous in presence and economically important viral disease of chickens caused by avian infectious bronchitis virus (IBV). In Indian subcontinent, prevalence of IBV was first reported in 1964[2] and the most prevalent form of IBV is respiratory form related to the Massachusetts strain M41and nephropathogenic IBV[3,4,5,6]. IBV is the prototype species in the family *Coronaviridae,* genus *Gammacoronavirus,* order *Nidovirales.* IBV genome encodes for 3 major structural proteins, phosphorylated nucleoprotein (N), membrane protein (M) and the large glycosylated spike protein (S)[7]. N and S proteins are major antigenic protein of IBV; S1 subunit of S protein contains epitopes for virus- neutralization and haemagglutination-inhibition[8]. The M glycoprotein is partially exposed at the surface of the virion and is of 678 nucleotides in length and a major type II integral membrane protein. M glycoprotein is essential for the production of coronavirus-like particles[7], whereas

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Coronaviruses have a minor type III envelope (E) protein of 330 nucleotides and the hydrophobic domain of IBV E protein alters the host secretory pathway and is important for release of infectious virus[8,9]. For genotypic characterization highly variable S1 gene based phylogeny are routinely employed [10, 11]. In this study we amplified, cloned and sequenced M and E gene of Indian IBV vaccine strain and characterized it based on M and E gene phylogenetic analysis.

MATERIALS AND METHODS

Virus

Viral RNA for cDNA preparation of M, E and S gene was isolated from avian infectious bronchitis vaccine, living bp (vet) (Mass type strain, Ventri Biologicals, Vaccine Division).

RNA isolation and RT-PCR

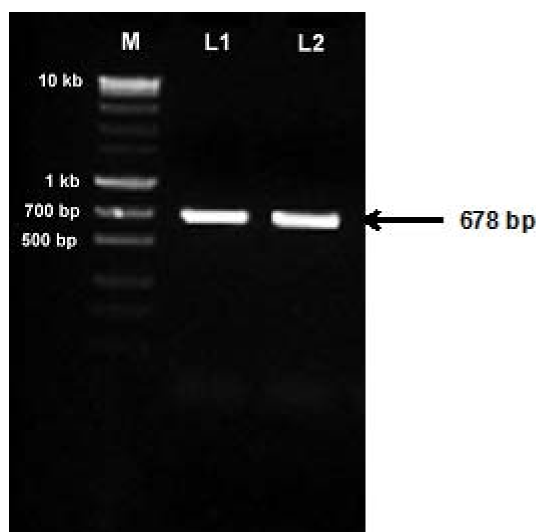
Total RNA from the reconstituted virus was extracted by Trizol reagent (Sigma, St. Louis, USA), as per the manufacturers protocol. RNA was eluted in 50ul elution buffer. The extracted RNA was first reverse-transcribed with gene specific primers and Thermoscript RT kit (Invitrogen,Carlsbad, USA) to synthesize the first strand cDNA. Primers used to amplify the M gene was forward 5' GTCGACATGTCCAAC GAGACAAATTG 3' (SalI) and reverse primer 5'AAGCTTTTAGGTGTAAAGACTACTCCCTC3' (HindIII) and E gene forward 5'GCGGGATCCAT GATGAATTTATTGAATAAG3' (BamHI) and reverse 5'GCGAAGCTTTCAAGAGTACAATT TGTCTC3' (HindIII) designed using whole genome of IBV strain FJ904723 to amplify the M and E gene complete cds. PCR amplification was carried out using the following cycling conditions: 94C for 5 min followed by 30 cycles of 94C for 1 min, 60C for 1 min, 72C for 1.5 min, with a final extension of 72 C for 10 min. The PCR amplified gene fragment was cloned into a T/A cloning vector pTZ57R/T (MBI Fermentas, Germany). The size of amplicon was 678 bp (M gene) and 330 bp (E gene). PCR product was visualised by agar gel electrophoresis and confirmed by restriction enzyme digestion and sequencing.

Sequence and phylogenetic analysis

Sequences were analysed using Chromas Lite 2.1.1. The nucleotide sequences of the M and E gene of Indian IBV vaccine strain were assembled, aligned by clustalW[12] after BLAST search with published IBV sequences deposited in the GenBank database. Sequence identities by BLAST analysis were included in the alignment and phylogenetic tree construction. The multiple sequence alignments and phylogenetic tree construction with neighbour joining method were performed using MEGA version 5. The bootstrap values were determined from 1000 replicates of the original data. Phylogenetic analysis of nucleic acid and deduced amino acid sequences was done with the neighbour-joining method using the Jukes-Cantor model and pairwise deletion. Reported M and E gene sequences of commonly used IBV vaccine H120, H52, Ma5, M41, China vaccine strains W93, H94, D41, IBN, HK and Mass-type was taken for phylogenetic analysis.

RESULTS AND DISCUSSION

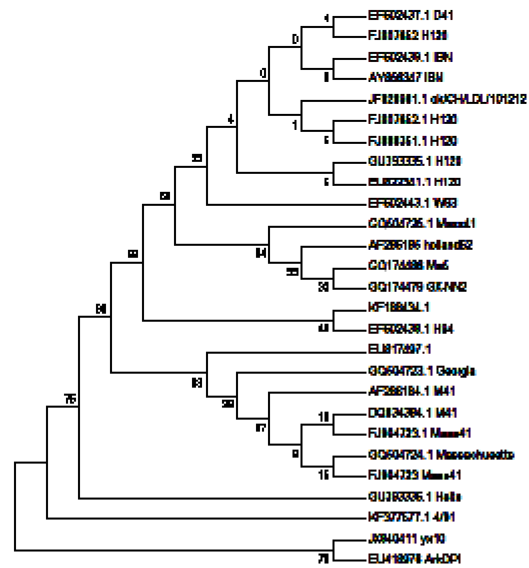
In the present study, M and E gene of Indian vaccine strain, Avian IBV living bp (Ventri Biologicals, Vaccine Division, Pune, India) routinely used for commercial vaccination was amplified, cloned in pTZ57R/T and sequenced. On agarose gel electrophoresis product of 678 bp and 330 bp was observed corresponding to M and E gene of IBV (Fig 1 and 2). Amplified PCR products was gel purified, cloned and sequenced by Sanger sequencing. Resulting sequence of M and E gene



Lane M: Mass Ruler Express Forward DNA marker
Lane L1: PCR amplicon

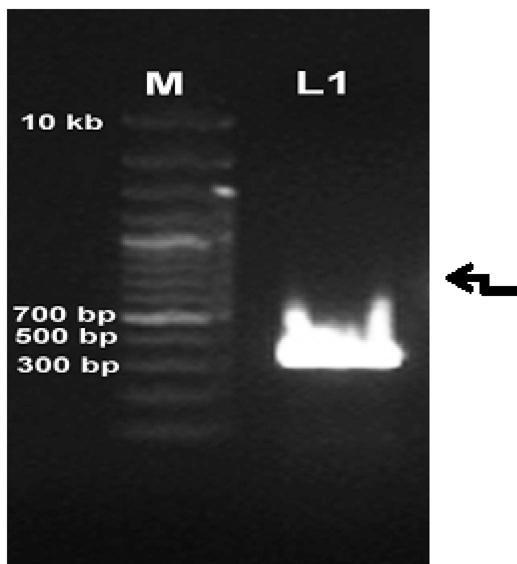
Fig. 1. PCR amplification of M gene from IBV

was submitted to GenBank under the accession number KF188434 (M gene) and KF188435 (E gene). BLAST analysis of M and E gene sequence confirms that it belongs to avian IBV. Phylogenetic analysis was performed for identification of Indian vaccine strain genotype. Phylogenetic tree constructed by aligning M gene sequence with routinely used vaccine strain and field isolate sequence shown that sequenced Indian IBV vaccine belongs to clade containing vaccine strain H120, H52, Ma5, M41, Chinese vaccine strains W93, H94, D41, IBN, HK (Fig. 3) and there was close association between Indian vaccine strain and H94 Chinese vaccine strain. Tree topology also shown that Indian Vaccine strain share common ancestor with H120, H52, Ma5, M41, Chinese vaccine strains W93, H94, D41, IBN, HK. One Scientist have also performed genotyping and characterization of Chinese strain HH06 based on membrane gene-phylogenetic analysis[13]. E gene phylogenetic tree constructed also shown similar results compared to M gene tree (Fig. 4). The sequences of the M gene showed 96.8-99.7% homologous identity at nucleotide level with published reference vaccine strain and field isolate strains from different regions and countries with minimum percent identity 96.8% with M41 strain (DQ834384) and maximum identity with H94 vaccine strain (EF602438). Whereas E gene showed 82.1-100% homologous identity at nucleotide level with published reference vaccine strain and field isolate strains from different regions and countries with minimum identity 82.1% with KF377577 a 4/91 strain and maximum identity 100% with H120 vaccine strain (EU822341, FJ807652, FJ888351 and



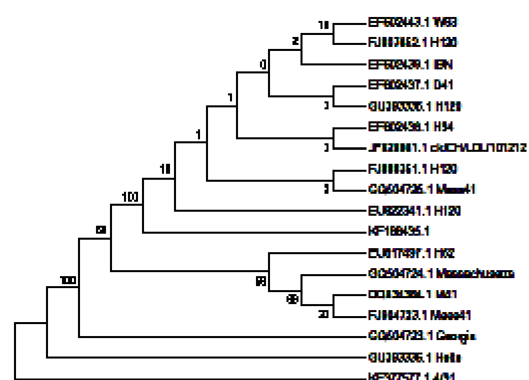
The tree was constructed with the neighbour-joining method (Jukes-Cantor method) with 1000 bootstrap replicates

Fig. 3. Phylogenetic analysis of the full-length cds of IBV M genes from Indian vaccine strain at the nucleotide level



Lane M: Mass Ruler Express Forward DNA marker
Lane L1: PCR amplicon

Fig. 2. PCR amplification of E gene from IBV



The tree was constructed with the neighbour-joining method (Jukes-Cantor method) with 1000 bootstrap replicates

Fig. 4. Phylogenetic analysis of the full-length cds of IBV E genes from Indian vaccine strain at the nucleotide level.

GU393335). IBV genotyping is routinely performed based on S1 gene phylogeny which is major variable and antigenic gene in IBV genome (Lee et al., 2001), here we have shown that conserved M and E gene based phylogenetic tree analysis can also show similar results compared with S1 gene sequence phylogeny. In conclusion M and E gene based characterization of IBV strain and isolate is easy and rapid method for characterization but as antigenic variations are concentrated within S1 region of Spike gene, analysis based on S1 gene will be more confirmatory.

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