DEVELOPMENT OF NUCLEIC ACID PROBES FOR THE DIAGNOSIS OF CHICKEN ANEMIA IN POULTRY

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Introduction

Chicken anaemia virus (CAV) is a single positive stranded DNA virus, which transiently causes severe anaemia and immunodeficiency in young chicks and subclinical in adults). Conventionally, diagnosis of CAV infection can be conducted by virus isolation of the detection of CAV-specific antibodies. However, these methods are cumbersome, less sensitive and time consuming that impairs the rapid and reliable laboratory diagnosis with clinical samples. An alternative diagnostic approach, polymerase chain reaction (PCR) technique, is known to be an extremely sensitive assay. Combination of PCR technique with ELISA technique will further improve the reliability of the diagnostic method. This research was designed DNA probes together with a reliable diagnostic process for the diagnosis and screening of chickens infected by CAV.

Materials and Methods

Chickens infected by CAV were identified by the use of ELISA. Infected chickens were sacrificed and thymus and liver were collected. These tissues were used as source of CAV DNA. Three pairs of oligonucleotide primers were designed and synthesised. These primers were used in PCR to specifically amplify CAV genes: VP1, VP2 and VP3 genes. These genes were cloned individually into plasmid vectors and subjected for DNA sequencing. The homology of these genes with various CAV strains were determined by sequence alignment computer software. DNA sequences that mostly unique to individual genes and the virus were determined. The DNA sequences selected were used to design a single unit of DNA probe namely dVP1, dVP2 and dVP3. The design could be expanded in combination with two or three units of probes of individual VP1, VP2 and VP3 genes. A single unit of individual probes was synthesised. A bigger single unit of the probe containing at least 50 tandem-repeats was constructed by the standard cloning method by using plasmids. Such recombinant plasmid could be used as a probe for detection of multiple short DNA targets by means of ELISA. Single stranded DNA probe could be prepared in a phagemid vector. The DNA target must first be obtained by the use of any PCR primers that can be used to amplify CAV DNA spanning the VP1, VP2 or VP3 gene.

Results and Discussion

Three sets of primers have been designed and found to be specific for chicken anaemia virus (Ghrissi and Mohd-Azmi, 1998). The entire VP1, VP2 and VP3 genes of CAV have been cloned into bacteria plasmid vectors (Raha et al. 1997). The VP3 gene has been fully sequenced and deposited into GENEBANK. The size of the VP3 gene was determined tob 372 bp and its DNA sequence could be accessed with accession number AF030518 (Mohd-Azmi et al. 1997). The VP3 gene has been further cloned in eukaryotic expression vector. In related studies, the VP3 gene has been expressed in bacteria and mammalian cell lines. It showed that the VP3 gene has anticancer properties which were potentially not limited to avian cells only. The VP2 and VP3 genes cloned have also been sequenced. The DNA sequences of the three genes have been analysed for the preparation of DNA probes. Single unit DNA probes were prepared for the individual genes. Results showed that the single unit probe dVP1 against VP1 gene was highly specific compared to the other two probes. However, combination of two probes, dVP1 with either dVP2 or dVP3, promotes both specificity and sensitivity. The single unit probe dVP1 was used to prepare tandem array of single-stranded DNA probe. Tandemly repeated DNA units of different length were successfully prepared by ligation method with DNA ligase and cloning. Further treatment and self-ligation to identical units increased the overall length of the probe. Various sizes of probes were cloned into plasmid pSPORT. Bacteria *Escherichia Coli* JM109 were transformed with the recombinant plasmid and co-infected with phage M13KO7. Phage particle containing ssDNA probe were purified and used as an immobilised probe in ELISA plate. The probe was then hybridised to the solid phase by means of uv-cross-linking. The immobilised probes were reacted with PCR products that contained the specific portion VP1 gene. Primers used in the PCR were labeled with biotin. Results of such hybridisation showed that the maximum signal as detected in ELISA could be reached when the number of tandem repeats per unit probe was reaching approximately 50 copies. The signal was not significantly improved when the number of repeats further increased. Furthermore, when the length of probe was increased further, the instability recombinant plasmid and decreased in recovery of ssDNA were observed. The detection limit is defined as the minimum amount of PCR amplified product of target samples required for the detection system to work and its is very minimum i.e., 1 ng. The technique developed here is simple and highly sensitive to detect PCR-amplified of CAV DNA samples.

Conclusions

The DNA probe developed is very sensitive and useful for the development of a full working diagnostic kit for chicken anaemia virus infection. The use of an appropriate DNA binding protein to bind the DNA probe to the ELISA plate would be of value for the preparation of low-cost diagnostic kit.

References

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