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Identification and analysis of putative promoter motifs in bovine herpes virus

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Abstract:
The purpose of this study is to identify and analyse the putative promoter motifs in the bovine herpes virus which causes several diseases in cattle worldwide including bovine mastitis with large economic impact on dairy industry. Bovine mastitis caused due to virus is often neglected as bacterial infections are held mainly responsible for the disease. Therefore, in this *in silico* investigation with all the existing experimental data a total of 147 promoter were identified along with their sequences from three genome viz bovine herpes virus 1 (BHV), bovine herpes virus 4 and bovine herpes virus 5, out of which 39 promoters were from bovine herpes virus 4 (BHV 4), 95 from BHV1 and 13 from BHV5 and it was observed that BHV1 and BHV5 have a close evolutionary history. However, they belong to the same subfamily and size of the genome and GC% of BHV1 and BHV5 was almost equal and very high compare to that of BHV4. This analysis may help in designing the live attenuated vaccine against BHV causing bovine mastitis that reduces the incidence of bovine mastitis. Identification of promoters may also help in designing of expression vectors which help in better understanding of the regulation of gene expression. In the era of large genomics and proteomics prediction of promoters in the whole genome is crucial for the advancement of drug discovery and gene therapy.

Keywords: Bovine herpes virus, bovine mastitis, promoters, transcription factors, genome.

Background:
Bovine mastitis is frequently occurring disease affecting dairy cattle with large economic losses in dairy industries. Though bacteria are the main causative agents of bovine mastitis, still there are 20 – 35% cases of bovine mastitis which are bacteriologically negative for the milk samples **[1, 2]** perhaps there may be pathogens other than bacteria causing disease, reports suggests that bovine herpes virus 4 is isolated from the milk of cows with mastitis **[3].** Bovine herpes virus 4 is also isolated from the milk of staphylococcus mastitis. Reports suggests that there is a positive association between the bovine herpes virus 4 seropositivity of cows and the incidence of bovine mastitis caused by *S. aureus* **[4]** and *S.aureus* is found to be the main causative agent of bovine mastitis **[5].**

Bovine herpes virus 4 is a member of the family herpesviridae, subfamily gamma herpesvirinae belonging to the order herpesvirales and genes rhadinovirus. BHV 4 has an enveloped icosahedral nucleocapsid, with a diameter of 100 nm, while the overall diameter of the virus particle is approximately 150 nm. The present study was also extended for the analysis of the genome of BHV1 and BHV5 which belongs to order herpesvirales family herpesviridae, subfamily alphaherpesvirinae, genus varicellovirus. BHV1 and BHV5 are held responsible for causing the most important emerging diseases of dairy cattle in many countries of the world. The disease caused by BHV in dairy cattle is characterized by the signs of respiratory disorder, general illness, abortion and reduced quality and quantity of milk yield. Virus has a wide host range which is known to infect different ruminant species like cattle, sheep and goats **[6, 7].** BHV needs dividing cells for effective virus replication as there is increase in viral DNA replication and protein expression at the S phase of the cell cycle.

The regulation of gene expression can be controlled at transcription level by a promoter region that contains a specific DNA sequence from which transcription begins and proceeds through the coding sequences and ends at the terminator site. The sequence of the promoters affects the transcription initiation event and also influences the rate at which RNA polymerase clears the promoter region to begin elongation in a typical transcription process. Therefore, identification of promoter sequences in a whole genome is crucial for regulation of gene expression in BHV. However, a limited number of data is available on the BHV. Hence, the present study was carried out through a computational method for the identification and analysis of putative promoter motifs in BHV.

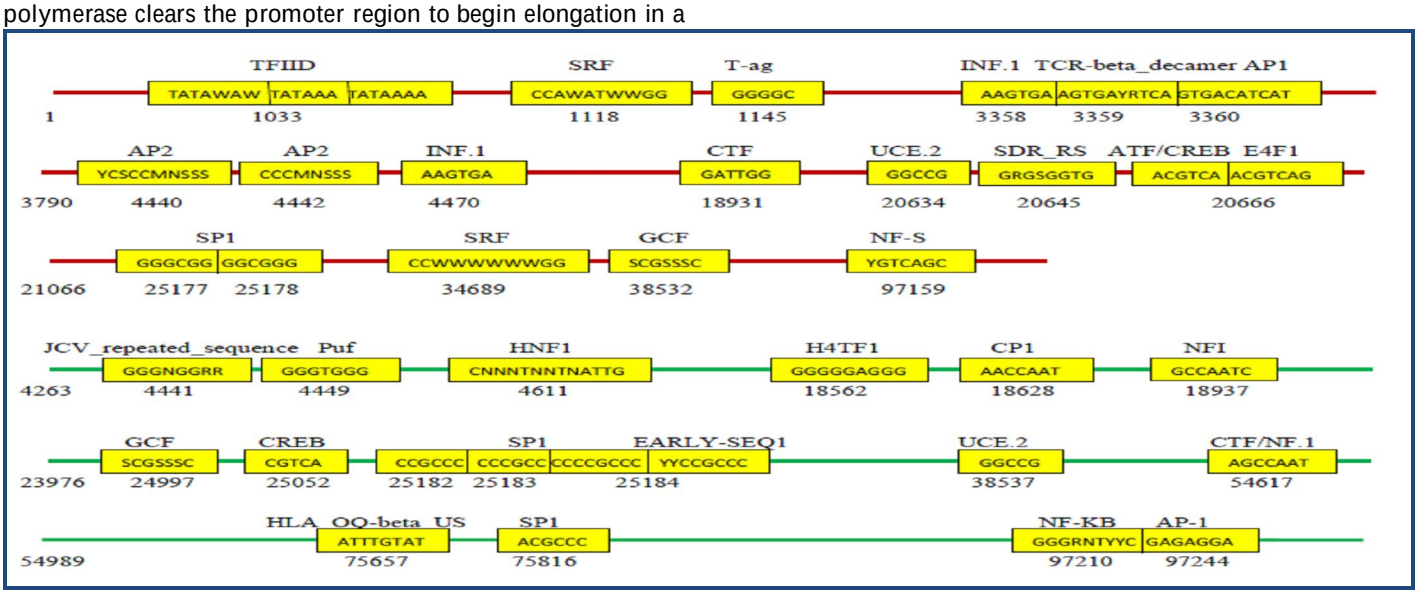

Figure 1: The promoters of Bovine Herpes virus 4.

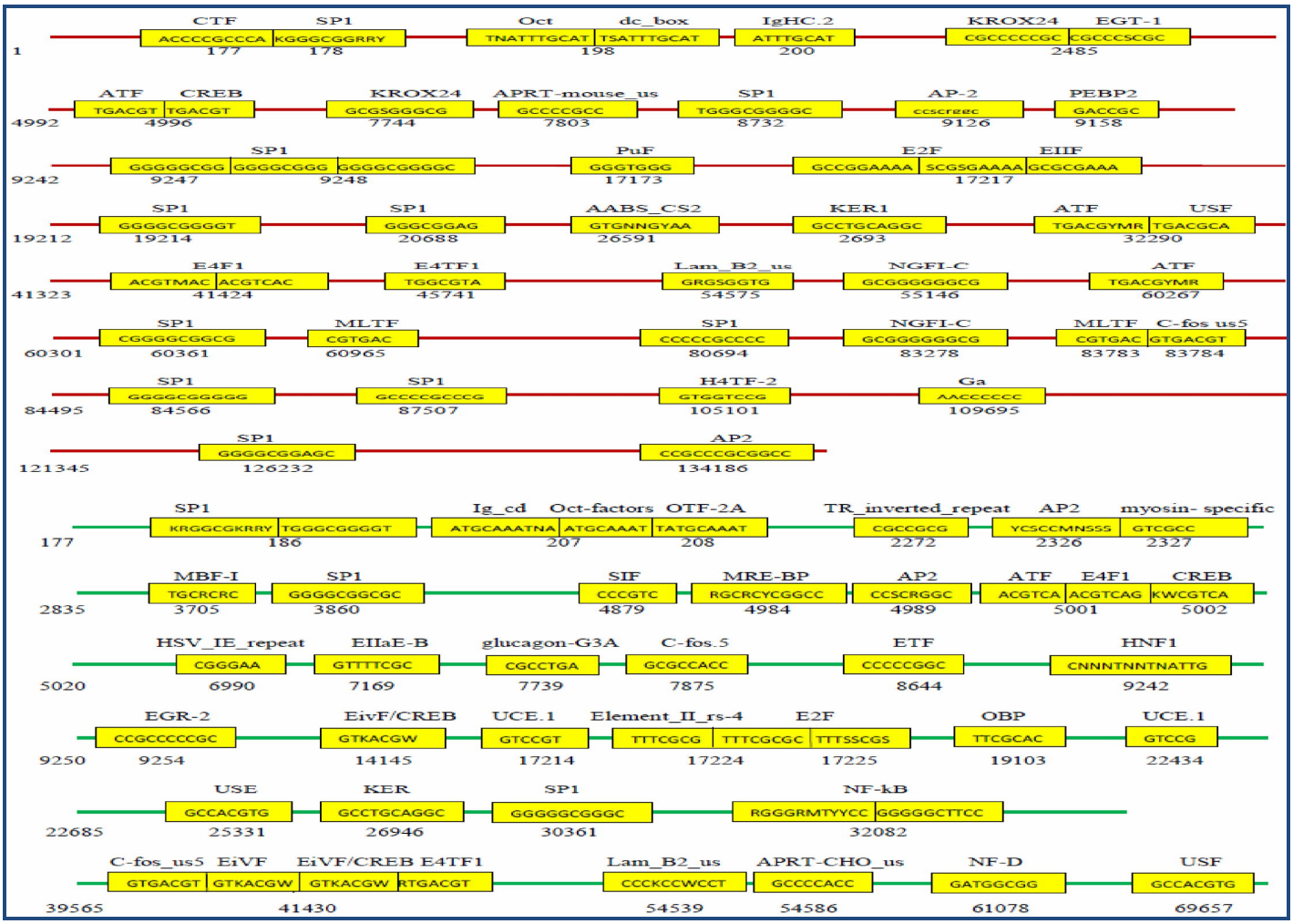

Figure 2: The promoters of Bovine Herpes virus 1.

Methodology

Retrieval of genome sequence

The complete genome sequences of BHV were retrived from biological database such as National centre for Biotechnology Information (NCBI) cited at http://www.ncbi.nlm.nih.gov/genomes/viruses.html.

Analysis of BHV genomes

The size of the genome of bovine herpes virus (BHV) was analysed in the FASTA format the G+C % was compared and total number of genes were also noted.

Transcription promoter site

The putative promoter in the genome of BHV was identified by using the promoter scan programme at http://www-bimas.cit.nih.gov/molbio/proscan/. The complete genome BHV1, BHV4 and BHV5 was used for the analysis of promoters in all three genomes. The program comprises three databases such as TF databases, promoter databases and non promoter set constructed from protein and RNA gene sequences. In this study we have provided a brief description of putative promoters of Bovine herpes virus. However, there are numerous methodologies available in the public domain for the analysis of promoters but still further validation is needed for a researcher before picking up the problem for investigation in the molecular biology laboratory.

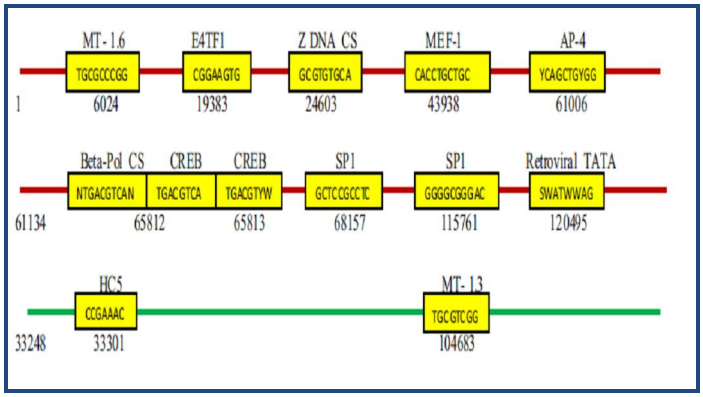

Figure 3: The promoters of Bovine Herpes virus 5. The red line indicates the positive strand; The green line indicates the negative strand; The yellow box indicates the promoter sequence and the number below the box indicates the specific location of the promoter. The letters above the box indicates the promoter designation.

Results and Discussion:

In the present study complete genome sequence of BHV 4, BHV1 and BHV5 was retrieved from the accession on NC_002665.1, NC_001847.1 and NC_005261.2 and the size of genome was found to be 108.87 kb, 135.3kb and 137.87 kb respectively the highest GC % was found in 74.8% in BHV 5 followed by BHV1 72.4% and BHV4 41.4% the highest number of genes were found in BHV 4 79 followed by BHV1 and BHV5 70 genes each. Similarly reports suggest that the genome size of Kamati River and Tick borne encephalitis virus was 11 kb, Tamana bat virus was 10,053 bases and GC% of Tamana bat virus and Louping ill virus was 38.43 and 54.85 respectively **[8].** DNA with high GC-content is more stable than DNA with low GC-content. GC base pairs are more stable than AU base pairs, due to the fact that GC bonds have 3 hydrogen bonds and AU only has 2 hydrogen bonds, which makes high-GC-content RNA structures more tolerant to high temperatures. More recently, the first large-scale systematic gene-centric association analysis demonstrated the correlation between GC content and temperature for certain genomic regions while not for others **[9].** Further, the identification of putative promoter in the genome of BHV1, BHV4 and BHV5 was also carried out. A total of 147 types of promoters and their sequences were identified in the genomes **(Fig 1, 2 and 3)** most of the promoters found in all 3 genomes are similar and it is confirmed with the existing data.

A very limited data is available on the identification and charecterization of promoter in the virus genome. Transcription factor IID (TFIID) is one of the general transcription factors that make up the RNA polymerase II preinitiation complex **[10]** TFIID binds to TATA box in the core promoter of the gene. It regulates the activities of more than 70 polypeptides required for the initiation of transcription by RNA polymerase II and also acts as channel for regulatory signals. SRF is a serum response element – binding transcription factor **[11]** that regulates the activity of many immediate early genes viz C-fos and thereby participates in cell cycle regulation, apoptosis, cell growth and cell differentiation. SV40 is a double stranded DNA virus which causes tumors at multiple sites to wide range of vertebrates. T- Ag is a protein of proto- oncogene present in the SV 40 and is involved in viral genome replication and regulation of host cell cycle **[12].**

AP1 (activating protein1) is a collective term referring to determine transcription factor composed of Jun, Fos or ATF subunit that bind to a common DNA site. Different AP1 factors may regulate different target genes and thus execute distinct biological functions **[13].** NF1 gene promoter harbours a hypomethylated CpG island. Hence, methylation changes may be involved in the development of different types of neurofibromas and malignant transformation **[14].**

The human neurotropic papovirus JCV contains sequences within the two 98 –bp tandum repeat which play a key role in glial – specific transcription of the early and late stage of viral promoter sites **[15].** TCR V beta promoter contains a highly conserved decamer homologous to cAMP response element (CRE). It has been shown that TCR beta – chain expression immediately activated cAMP. Such induction is likely to be mediated through V beta-CRE sequence because the inclusion of V- beta – CRE in a vector with minimum promoter (PB1 CAT2) conferred the cAMP inducibility of CAT activity **[16].**

The promoter of the early growth response gene (Egr-1) has been described to be activated by ionizing radiation it has been reported that a novel regulatory element in the human Egr-1 promoter is similar to a NF kappa-B binding site **[17].** HNF-1 beta forms a homodimer or a hetrodimer with HNF1 alpha and regulates various target genes. HNF1 beta mutations are rare and no functional analysis has been performed in conjuction with HNF1 alpha. HNF1 beta controls liver specific and bile acid related genes as it is expressed in the liver and biliary system **[18].**

Conclusion:

In the present study attempts were made to find the promoter sites and regulation of genes and their function in the genomes

with the available data by computational methods. A total of 147 promoters were identified from three bovine herpes virus genome out of which 39 promoters were from bovine herpes virus 4 (BHV 4), 95 from BHV1 and 13 from BHV5. The results of the present study may help in designing of the live attenuated vaccine by site directed mutagenesis in the promoter region which could be a permanent solution for the problem bovine mastitis due to Bovine herpes virus. This present study of promoters might also help in designing of expression vectors which helps in better understanding of regulation of gene expression. In the era of large genomics and proteomics prediction of promoters in the whole genome is crucial for drug discovery and gene therapy.

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