Expression of the G1 epitope of bovine ephemeral fever virus G glycoprotein gene by pET24-G1 recombinant construct in *Escherichia coli*

Pasandideh, R.[1]; Beigi Nassiri, M.T.[2] and Seyfi Abad Shapouri, M.R.[3]

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Abstract

Bovine ephemeral fever (BEF) is a viral disease of cattle and water buffalo seen in some provinces of Iran, generally southern and warm regions in recent years. The G glycoprotein is the main protective antigen of the bovine ephemeral fever virus (BEFV) and the target of anti-BEFV neutralizing antibodies. The aim of the present study was cloning and expression of the G1 epitope of BEF virus G glycoprotein gene in a prokaryotic system. For this purpose, the G1 epitope was cloned in a prokaryotic expression vector, pET-24a(+), under the control of the T7 promoter and subsequently the recombinant pET24-G1 construct was transformed into Rosetta strain of *Escherichia coli*. Expressed recombinant protein was analyzed using SDS-PAGE and immunoblotting methods. SDS-PAGE analysis showed that a protein with ~18 kDa molecular weight, consistent with the expected molecular weight of recombinant protein fused to 6xHis tag, was expressed. Immunoblotting analysis showed that the expressed G1 protein specifically reacted with a mouse polyclonal serum against BEFV. Thus, in this study the G1 protein of BEFV was successfully expressed by the pET24-G1 recombinant construct in *Escherichia coli*. Based on the results of this study, immunization and the efficacy of this product can be consider as a possible candidate for the production of subunit vaccine against the virus in animal models.

Key words: Gene, Bovine Ephemeral Fever Virus, Recombinant G1 protein, *Escherichia coli*

1- PhD Graduated of Animal Genetics and Breeding, Faculty of Animal & Food Science, Khuzestan Agricultural Sciences and Natural Resources University, Mollasani, Iran

2- Professor, Department of Animal Science, Faculty of Animal & Food Science, Khuzestan Agricultural Sciences and Natural Resources University, Mollasani, Iran

3- Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Corresponding Author: Beigi Nassiri, M.T., E-mail: mt_nassiri@yahoo.com

Refrences

Aziz-Boaron, O.; Leibovitz, K.; Gelman, B.; Kedmi, M. and Klement, E. (2013). Safety, immunogenicity and duration of immunity elicited by an inactivated bovine ephemeral fever vaccine. PloS one, 8(12): 1-9.

Beigi Nassiri, M.T.; Pasandideh, R. and Seyfi Abad Shapouri, M.R. (2016). Cloning and expression of the G1 epitope of bovine ephemeral fever virus G glycoprotein in *Escherichia Coli*. Genetics in the 3rd millennium, 14(2): 4250-4255.

Cybinski, D.; Davis, S. and Zakrzewski, H. (1992). Antigenic variation of the bovine ephemeral fever virus glycoprotein. Archives of virology, 124(3-4): 211-224.

Dhillon, J.; Cowley, J.A.; Wang, Y. and Walker, P.J. (2000). RNA polymerase (L) gene and genome terminal sequences of ephemeroviruses bovine ephemeral fever virus and Adelaide River virus indicate a close relationship to vesiculoviruses. Virus research, 70(1): 87-95.

Hertig, C.; Pye, A.D.; Hyatt, A.D.; Davis, S.S.; McWilliam, S.M.; Heine, H.G. et al. (1996). Vaccinia virus-expressed bovine ephemeral fever virus G but not GNS glycoprotein induces neutralizing antibodies and protects against experimental infection. Journal of general virology, 77(4): 631-640.

Johal, J.; Gresty, K.; Kongsuwan, K. and Walker, P.J. (2008). Antigenic characterization of bovine ephemeral fever rhabdovirus G and GNS glycoproteins expressed from recombinant baculoviruses. Archives of Virology, 153(9): 1657-1665.

Kato, T.; Aizawa, M.; Takayoshi, K.; Kokuba, T.; Yanase, T.; Shirafuji, H. et al. (2009). Phylogenetic relationships of the G gene sequence of bovine ephemeral fever virus isolated in Japan, Taiwan and Australia. Veterinary Microbiology, 137(3): 217-223.

Kongsuwan, K.; Cybinski, D.H.; Cooper, J. and Walker, P.J. (1998). Location of neutralizing epitopes on the G protein of bovine ephemeral fever rhabdovirus. Journal of General Virology, 79(11): 2573-2581.

Pasandideh, R.; Beygi Nassiri, M.T.; Seyfi Abad Shapouri, M.R.; Fayazi, J.; Roshanfekr, H. and Lotfi, M. (2016). Cloning and sequencing of G glycoprotein gene of bovine ephemeral fever virus in Escherichia coli. Iranian Journal of Animal Science Reaserch, 8(3): 511-519 (in persian).

Qiagen (2001). The QIAexpressionist. In Handbook for High-Level Expression Purification of 6xHis-Tagged Proteins. Hilden, Germany, 1-125.

Ramos, C.R.R.; Abreu, P.A.E.; Nascimento, A.L.T.O. and Ho, P.L. (2004). A high-copy T7 *Escherichia coli* expression vector for the production of recombinant proteins with a minimal N-terminal His-tagged fusion peptide. Brazilian Journal of Medical and Biological Research, 37(8): 1103-1109.

Terpe, K. (2003). Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. Applied Microbiology and Biotechnology, 60(5): 523-533.

Trinidad, L.; Blasdell, K.R.; Joubert, D.A.; Davis, S.S.; Melville, L.; Kirkland, P.D. et al. (2014). Evolution of bovine ephemeral fever virus in the Australian episystem. Journal of Virology, 88(3): 1525-1535.

Uren, M.F.; Walker, P.J.; Zakrzewski, H.; St George, T.D. and Byrne, K.A. (1994). Effective vaccination of cattle using the virion G protein of bovine ephemeral fever virus as an antigen. Vaccine, 12(9): 845-852.

Walker, P.J. (2005). Bovine ephemeral fever in Australia and the world. Current Topics in Microbiology and Immunology, 292: 57-80.

Walker, P.J. and Klement, E. (2015). Epidemiology and control of bovine ephemeral fever. Veterinary Research, 46(1): 1-17.

Wallace, D.B. and Viljoen, G.J. (2005). Immune responses to recombinants of the South African vaccine strain of lumpy skin disease virus generated by using thymidine kinase gene insertion. Vaccine, 23(23): 3061-3067.

Zheng, F.Y.; Lin, G.Z. and Qiu, C.Q. (2007a). Expression, purification and antigenic characterization of the epitope-G1 gene of bovine ephemeral fever virus in *Escherichia coli*. Acta microbiologica Sinica, 47(3): 498-502.

Zheng, F.Y. and Qiu, C. (2012). Phylogenetic relationships of the glycoprotein gene of bovine ephemeral fever virus isolated from mainland China, Taiwan, Japan, Turkey, Israel and Australia. Virology Journal, 9(1): 1-8.

Zheng, F.Y.; Lin, G.Z..; Qiu, C.Q.; Yuan, K.Z. and Song, J.Y.. (2007b). Expression and antigenic characterization of the epitope-G1 of the bovine ephemeral fever virus glycoprotein in *Pichia pastoris*. Virologica Sinica, 22(5): 347-352.

Zheng, F.Y.; Lin, G.Z.; Qiu, C.Q.; Zhou, J.Z.; Cao, X.A. and Gong, X.W. (2009). Development and application of G 1-ELISA for detection of antibodies against bovine ephemeral fever virus. Research in Veterinary Science, 87(2): 211-212.

Zheng, F.Y.; Lin, G.Z.; Qiu, C.Q.; Zhou, J.Z.; Cao, X.A. and Gong, X.W. (2010). Serological detection of bovine ephemeral fever virus using an indirect ELISA based on antigenic site G 1 expressed in *Pichia pastoris*. The Veterinary Journal, 185(2): 211-215.