



Supplementary Material

In silico Analysis of Non-Synonymous Single Nucleotide Polymorphisms in α -Toxin of *Clostridium perfringens* Toxinotype B Isolated from Lamb Dysentery Cases in Pakistan

Mian Muhammad Khubaib Sattar^{1,4}, Aftab Ahmad Anjum^{1*}, Yung Fu Chang², Tahir Yaqub¹ and Asim Aslam³

¹Institute of Microbiology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, 54000 Lahore, Pakistan.

²Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, 14853 Ithaca, New York, United States of America.

³Department of Pathology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, 54000 Lahore, Pakistan.

⁴Department of Microbiology, Faculty of Veterinary and Animal Science, The Islamia University of Bahawalpur, 63100 Bahawalpur, Pakistan.

SUPPLEMENTARY MATERIAL

The current study is in continuation to our previously published research, [Sattar, M.M.K., Anjum, A.A., Chang, Y.F., Yaqub, T., Aslam, A. and Ali, T., 2023. Molecular Characterization and toxins optimization of indigenous *Clostridium perfringens* Toxinotype B isolated from lamb dysentery clinical cases. *Kafkas Univ Vet Fak Derg* **29**: 79-89. <https://doi.org/10.9775/kvfd.2022.28738>] in this article the *in silico* gene expression analysis was conducted on the α -toxin gene of *C. perfringens* toxinotypes B.

Gene amplification and sequence for SNP annotation

The α -toxin (*cpa*) gene of *Clostridium perfringens* toxinotype B was amplified by using primer sequence CPA-F 5'-GCTAATGTTACTGCCGTTGA-3' CPA-R 5'-CCTCTGATACATCGTGTAAG-3' and amplified at 94°C for 10 min (initial denaturation) followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 45 sec, extension at 72°C for 30 sec and final extension

at 72°C for 10 min. Gel electrophoresis was performed using 1.8% agarose with ethidium bromide 0.5 µg/mL. The amplified gene product was sequenced by Sanger chain termination sequencing which retrieved following nucleotide sequence; CCTGCTGTTCTTTTGAGAGT-TAGCTAAAGTTACCTTTGCTGCATAATCCCAAT-CATCCCACTATGACTCATGCTAGCATGACTATA-GTATATTGATTTTCCTGTTTTAGCAAAACCTCTTG-CATATTCTTTTGACCATGCATTAATAATCTTTGT-TTTTAAAGATATCAGCATAAAAATCCTCATTAGT-TTTCGCAACCTGCTGTGTTTATTTTATACTGTTCT-TTCTTTCTCTGCAAAAGTCTCAAACCTAACAT-GTCCTGCGCTATCAACGGCAGTAACATTAGCAAC.

* Corresponding author: aftab.anjum@uvas.edu.pk
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