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Genetic analysis of Beta 2 -toxin OF Clostridium perfringens type B and C strains

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SUMMARY

The present study reports the presence of the β_2 toxin gene cpb_2 in both C and B strains of C. perfringens but with a great degree of heterogeneity on the bases of nucleotides sequence. The gene was found associated with the chromosomal but not with the plasmid DNA. The DNA sequencing revealed that the difference in the nucleotide sequence found in the middle of the gene between nt 35 – 220 with the most diversity lies between nt 550 -700 downstream to 5' end of the gene. At nt 36 – 45, lies a pronounced divergence of the cpb_2 from B strain from the consensus sequence where C strain marked the divergence at nt 140 – 150. On the other hand there is a great diversity lies between B and C strains at the position of nt 105- 150 but all come with consensus sequence

INTRODUCTION

Clostridium perfringens is an endospore-forming, gram-positive anaerobic bacterium that ranks among the most important of the anaerobic pathogens affecting humans and domestic animals

Type B and C strains of C. perfringens cause necrotic enteritis primarily in pigs, chickens, cattle, sheep, and goats. Although adult animals can contract this disease, it most frequently occurs in the young of these species (Timoney et al., 1988). Piglets are particularly susceptible to type C infections (Johnson et al., 1992 and Taylor 1984), During a type C infection, necrosis of the intestine can be extensive and deaths appear to be the result of toxemia with beta toxin (Songer 1996 and Helen et al., 2005).

Infection of humans by type C strains appears to be largely restricted to certain tribal populations in Papua New Guinea, although infrequent cases of type C infection have occurred in humans throughout the world. Type C infections result in necrotizing enterocolitis ("pigbel") in these individuals after consumption of undercooked pork during certain ritualistic practices (Lawrence et al., 1990). The importance of beta-toxin in both animal and human disease has been demonstrated by immunization studies using a toxoid of beta-toxin. When immunized with the toxoid of beta-toxin, the Papua New Guinea tribespeople experienced a fivefold reduction in the incidence of necrotic enteritis (Shatursky et al., 2000), whereas a beta-toxin toxoid administered to infant pigs during an outbreak of necrotizing enterocolitis reduced mortality by approximately 30% . In the case of agriculturally important animals, vaccination against type C infections is universally advocated in order to avoid devastating losses. Therefore, beta-toxin plays a key role in the lethal outcome of type B and C infections.

The 28-kDa β_2 toxin was first purified from C. perfringens type C strain CWC245 and shown to be cytotoxic for Chinese hamster ovary cells (Jolivet-Reynaud et al., 1986) and that presence of cpb2 sequences in all types of C. perfringens was proven. The deduced amino acid sequence of CPB2 is highly conserved in type A and C strains of C. perfringens isolated from diarrheic pigs (Waters et al., 2003). However, the variability in CPB2 sequences in type A human isolates coupled with CPB2 sequence differences in isolates from nonporcine animals (Jost et al., 2005) indicate that CPB2 is an unusual C. perfringens toxin.

cpb2 encodes β_2 toxin, which was toxic to cultured epithelial cells and lethal to mice when administered intravenously (Gibert et al., 1997). cpb2-positive C. perfringens strains are associated with the occurrence of enteric disease in domestic animals, notably pigs (Bueschelet al., 2002 ,Garmory et al.,2000 , Klaasen et al.,1999 and Waters et al.,2003), horses (Bacciarini et al., 2003 and Herholz et al., 1999), and dogs (Thiede et al., 2001). There was an especially strong correlation between the prevalence of cpb2 in isolates from piglets with enteritis and the absence of cpb2 in isolates from healthy piglets thus strong correlation between β2-toxin phenotype and genotype was seen in type A and C isolates obtained from diseased pigs (Bueschel et al., 2002 and Moreno et al., 2003). The molecular mechanism elucidated for β_2 toxin which could be used as a basis for investigating its role in the pathogenesis of these clostridial pathogens remains under extensive study. It has been suggested that β_2 toxin may be a pore-forming toxin on the basis of weak similarities (10% identity) between the primary structure of β-toxin and

those of the pore-forming α-hemolysin and γ-hemolysin and the leukocidin from Staphylococcus aureus (Hunter et al ., 1993).

This study aimed to investigate the genotype analysis of b_2 toxin gene in C. perfringens type B and C strains as a preparatory step to produce DNA vaccine.

MATERIAL AND METHODS

Strains:

Overnight culture of C. Perfringens type B and C standard strains were injected S/C in guinea pigs (0.5mL) and re-isolated from the liver and heart blood of the dead guinea pigs on cooked meat agar. After 24 h of incubation at 37°C under anaerobic condition, a single colony was picked and propagated on cooked meat broth and incubated as before.

DNA Extraction:

The DNA was extracted from the 24h broth culture of both strains by Triazol method (Zhou et al., 2009) with some modification. Briefly, the culture was centrifuged at 14000rpm/10min/4°C; cell pellets were reconstituted in 1 ml TE buffer pH 8 and incubated at 37°C for 2 hours with 5ul lysozymes (final concentration 100µg/ml). Proteinase-K was added 50µl/1ml (final concentration 100µg/ml) and incubated for further 3 hours at 56°C with shaking then 1 ml of Triazol was added. After vortexing for 30 sec, 0.5 ml of chloroform was added and centrifuged for 10 min at 14000 rpm. The DNA in the interphase was precipitated with 0.5 ml of absolute ethanol, washed twice with 0.1M sodium citrate in absolute ethanol and finally redissolved in 50ul of 8 mM NaOH. The pH was then adjusted at 8 by adding 115 µl/ml HEPES (0.1 M). Two µl of RNAase were then added and incubated at 37°C for 1 hour. Purification of the genomic DNA was done using Wizard DNA clean up system (Promega), and visualized under U.V. transelumination after electrophoresis on 1% agarose. Hind III digested λ phage (with bands size of 23.1 - 9.1 - 6.5 - 4.3 - 2.3 - 2.0-1.0 – 750 – 564 – 200 - 125) was used as a DNA marker

Miniprep:

The plasmids were extracted from the 24h culture of C. Perfringens type B and C using BioSpin Plasmid DNA Extraction Kit (BioFlux- Bioer Technology Co., Ltd. Japan)

PCR amplification:

The β2 toxin of C. Perfringens was amplified using the primer β_2-F (5'- TTA ATA ACA ATA ACC CTC ACC AAA - 3') and β_2-R (5'-TGA AAA ATG ATA ATT AAA AAA GTA- 3') PCR was performed in 50-µl reaction mixtures containing 50 mM KCl, 10mM Tris-HCl (pH8.8), 3 mM MgCl2, 200 mM (each) deoxynucleoside triphosphate, 10µl Q solution (Qiagene) and 2.5 U of

thermostable Taq DNA polymerase and 50 pmol of each oligonucleotide primer. DNA samples (0.5 µg for genomic DNA and 1µg for plasmid DNA) were pipetted through into the mix true. Thermal cycling was performed using T gradient thermal cycler (Biometra, Germany), the parameters for amplification were denaturation at 95 °C for 3 min for one cycle and then 40 cycles at 95°C for 1 min (denature), 55-60 °C for 45 sec (gradient annealing) during the pilot test then the annealing temperature was adjusted at 60 °C/45 sec, and 72°C for 1 min (extension). A final extension at 72 °C for 10 min was also included. The amplicon was then visualized under U.V. transelumination after electrophoresis on 1% agarose. The size of the amplicons was analyzed in comparison to 100pb DNA ladder.

Sequencing:

The complete nucleotide sequences of the whole beta 2 toxin of both strains were performed in (Geospiza Inc, Koria). For preparation of the gene for sequencing, the PCR product was separated on 1% low melting agarose and electrophoresed on low voltage (20volt) at 4°C. The bands were sliced off and purified with the Biospin PCR purification kit as described by the manufacture. Briefly, the gel slices were melted at 60°C for 5 min, mixed with 500 ml of gel extraction buffer and placed on the biospin column provided with the kit, centrifuged at 4000xg/2min/4°C and washed twice with the washing solution. Finally the amplicon was eluted in 50 Ml of the elution buffer and stored at -20 °C till used

Analysis:

The sequence analyses including nucleotide alignments, deduced amino acid prediction and alignment and restriction mapping were performed using CLC Sequence Viewer Version 5.1.2 Windows vista 6.0 platform developed by CLC bio A/S

The PCR analysis was performed with gel pro, gel documentation system.

RESULTS

PCR amplification:

C. perfringens type B and C strains were subjected to both genomic and plasmid DNA extraction (Photo. 1) both gave a genomic DNA with apparent molecular size of 50 kpb while the plasmid DNA was about 11kpb.

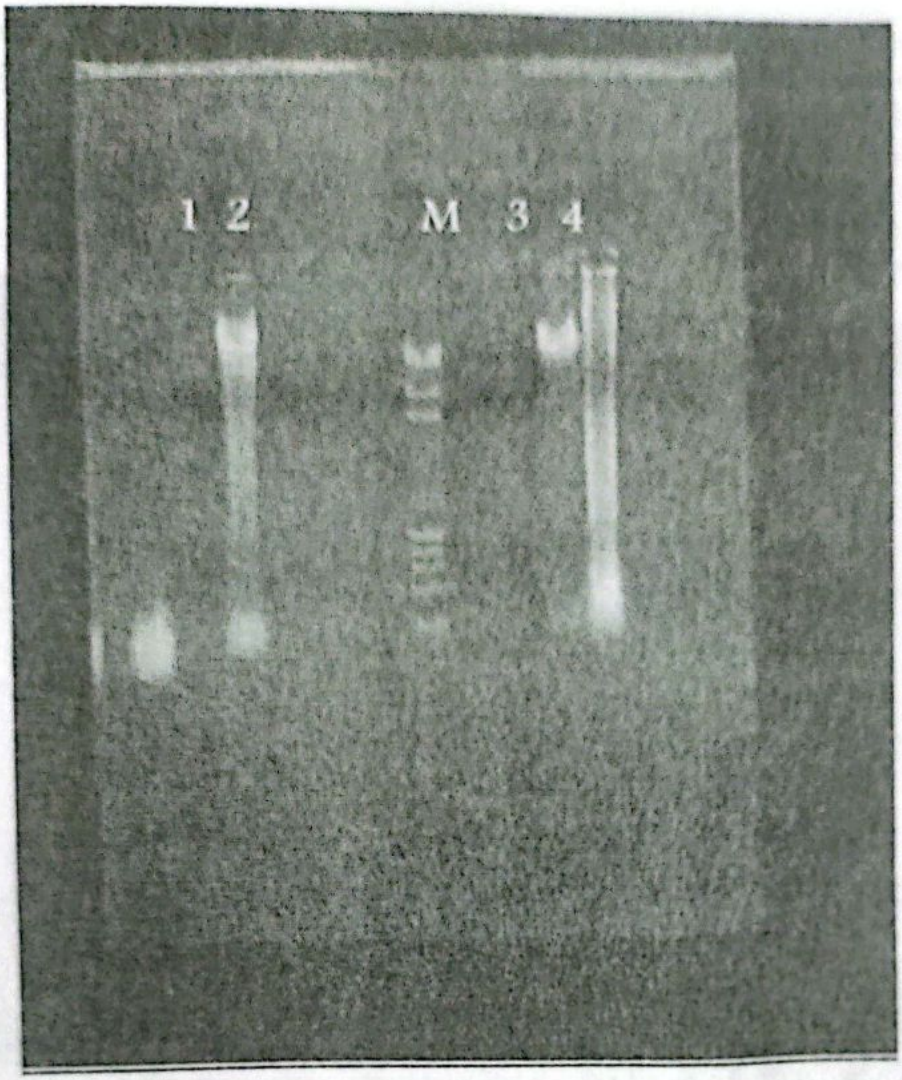


Photo (1) The plasmid and genomic DNA of C. Perfringens type B (lane 1and 2) and type C (lane 3 and 4). M: Hind III digested λ phage DNA marker

The Beta 2 toxin gene was amplified from both genomic and plasmid DNA, as shown in photo 2 lane 1 and 2, two bands of a proximate molecular size of 1344pb and 400 bp were detected with both strains. While using the plasmid DNA from both types as a template (even with different concentrations) no detectable amplification was noticed lane 3 to 7.

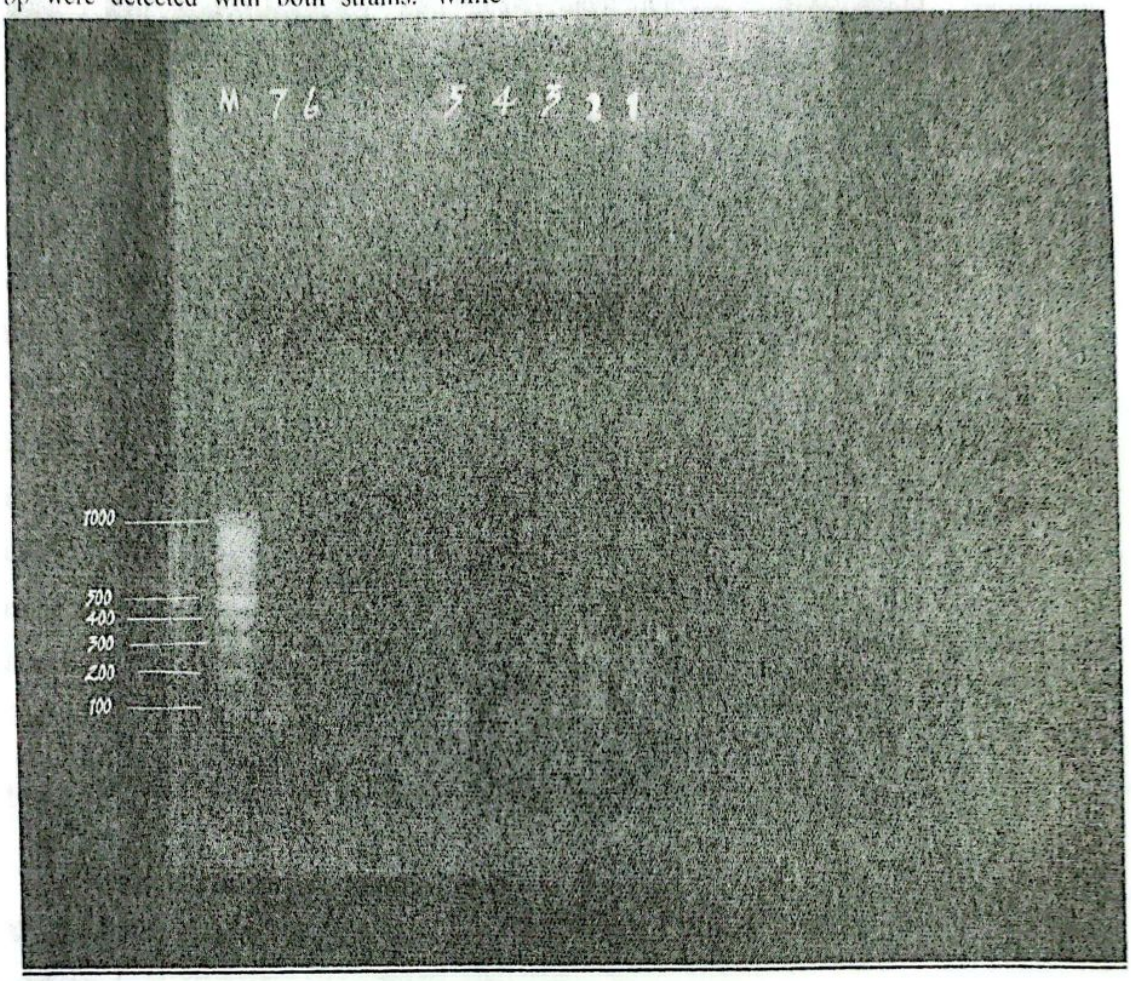


Photo (2) the PCR amplification of β2 toxin gene from the genomic DNA of C. perfringens type B (lane 1) and type C (lane 2), and the plasmid DNA (lane 3 to 7). Note the amplification of the 1344bp band corresponding to the full length gene plus a non specific band migrating at about 400 bp (annealing temperature was 60 °C) with the genmic DNA while no amplification was seen with the plasmid DNA.
M: 100 bp DNA ladder

Gene sequencing

The complete gene sequence of beta 2 toxin gene of C. perfringens, type B and C and the alignment are depicted in Figs 1 ,2 and 3 respectively.

The genetic analysis revealed a degree of heterogeneity between cpb_2 of B and C strain. The most significant finding in this study is the presentation of clear evidence that the sequence heterogeneity genuinely lies in the middle of the gene between nt 35 – 220 with the most diversity lies between nt 550 - 700 downstream to 5' end of the gene. At 36 – 45 lies a pronounced divergence of the cpb_2 from B strain from the consensus sequence

where C strain marked the divergence at nt 140 – 150. On the other hand there is a great diversity lies between B and C strains at the position of nt 105- 150 but all come with consensus sequence. This degree of heterogeneity is attributed to the differences in the restriction endonuclease map predicted when analyzing with enzymes that make either one or two cuts all over the entire gene (fig 5). All nucleotide difference in the coding region is base substitutions as there is except 3 areas with base deletions were observed with type B (position 36 – 44 , 586 – 596 and 608 – 615) and 2 areas with base deletions seen with type C(position 139 – 150 and 687 – 694). Concerning amino acid sequence prediction, a very pronounced difference was found at 33 – 40 amino acid where the YIYIYI sequence of C strain replaced with the SSYSTR in the B strain

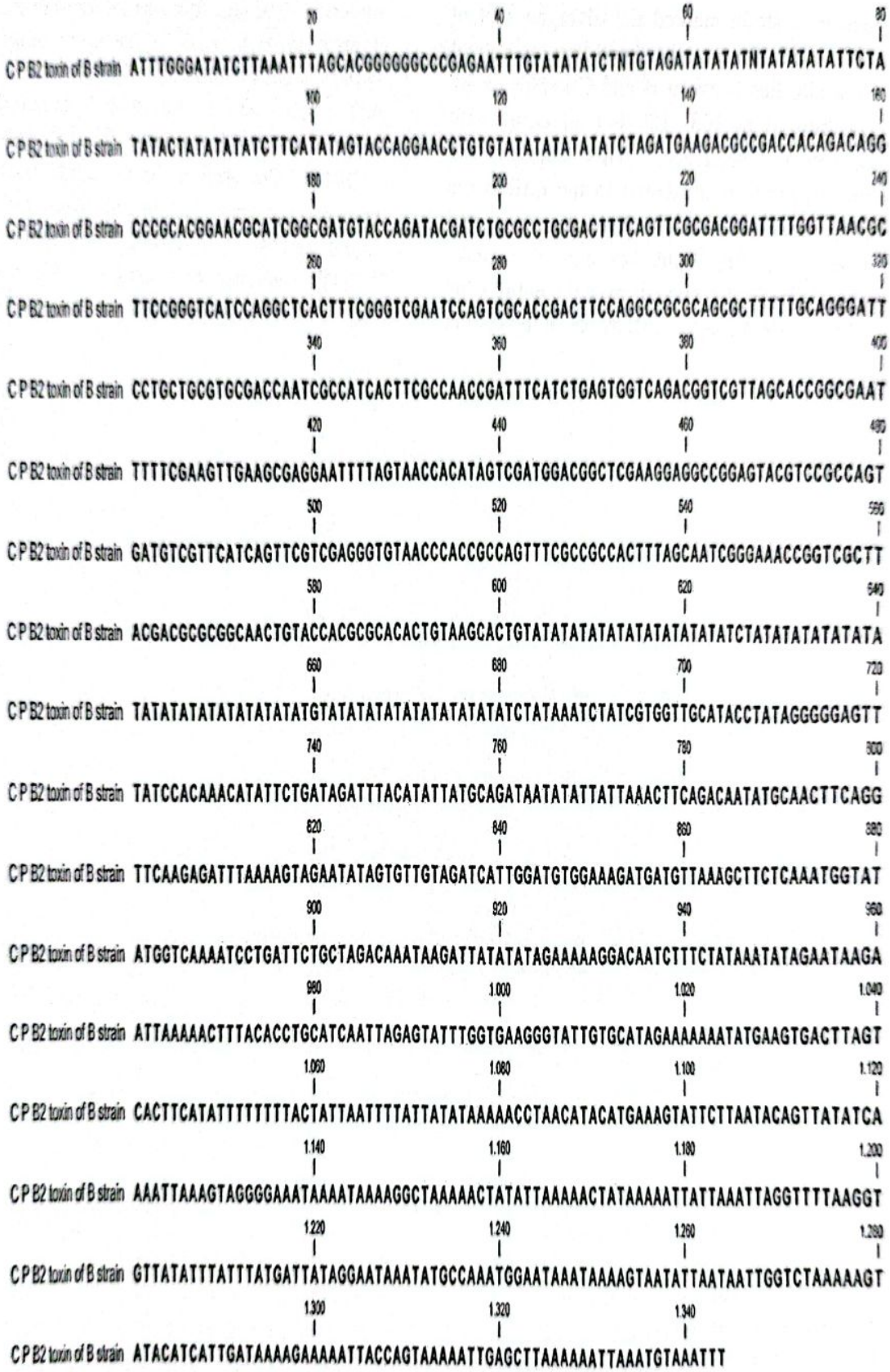
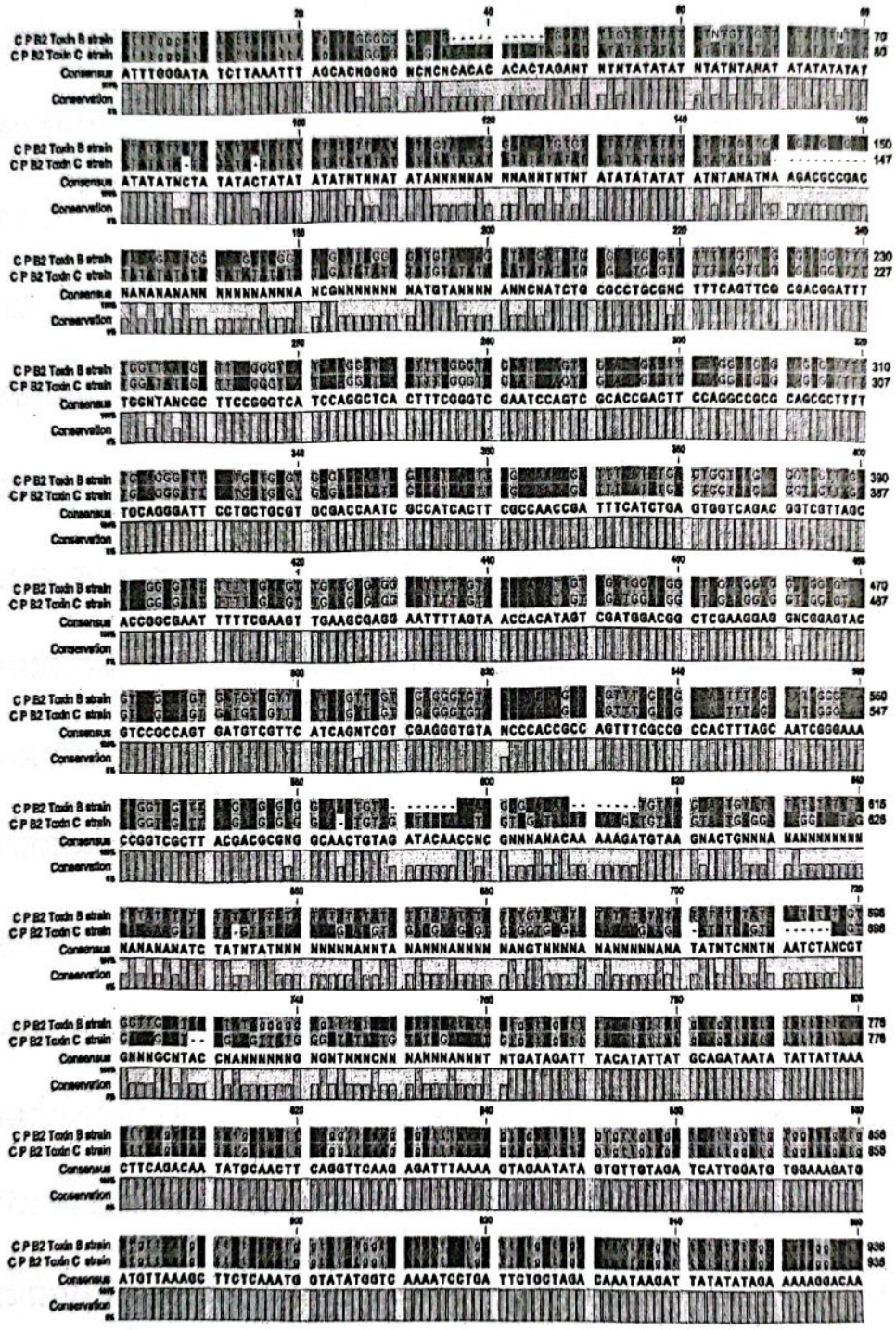


Fig (1) the complete nucleotide sequence of β_2 toxin gene of C. perfringens type B.

Fig (2) the complete nucleotide sequence of β_2 toxin gene of C. perfringens type C.



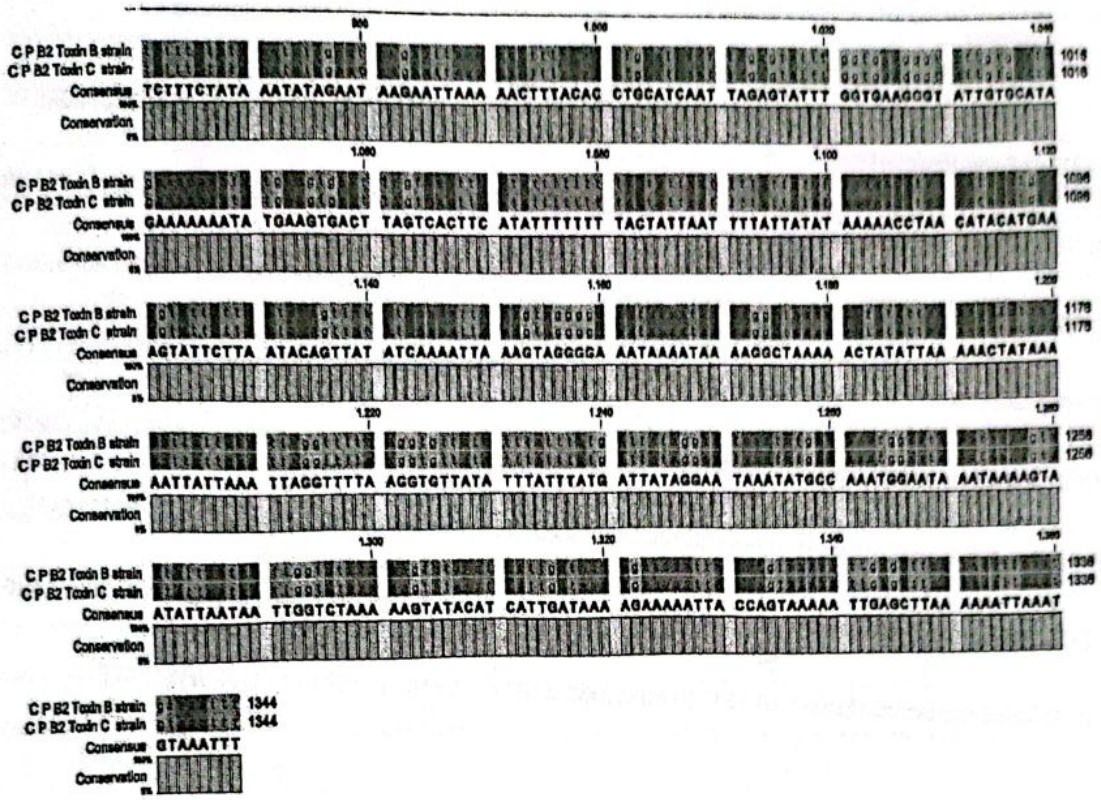


Fig (3) the alignment of the complete nucleotide sequence of β_2 ***Cl. perfringens*** type B and C strains

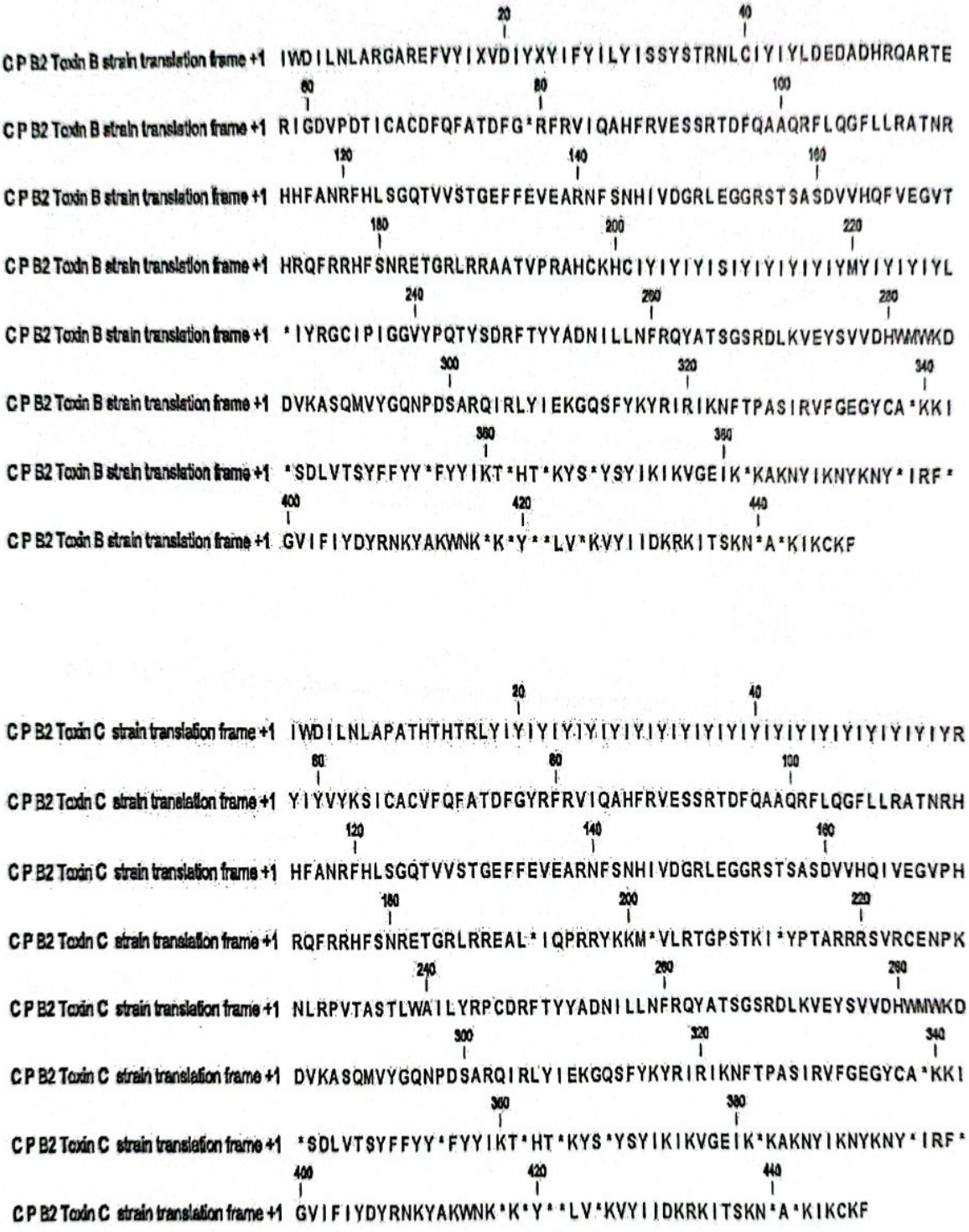


Fig (4) the dedicated amino acid sequence of β_2 toxin of C. perfringens type B and C strains.

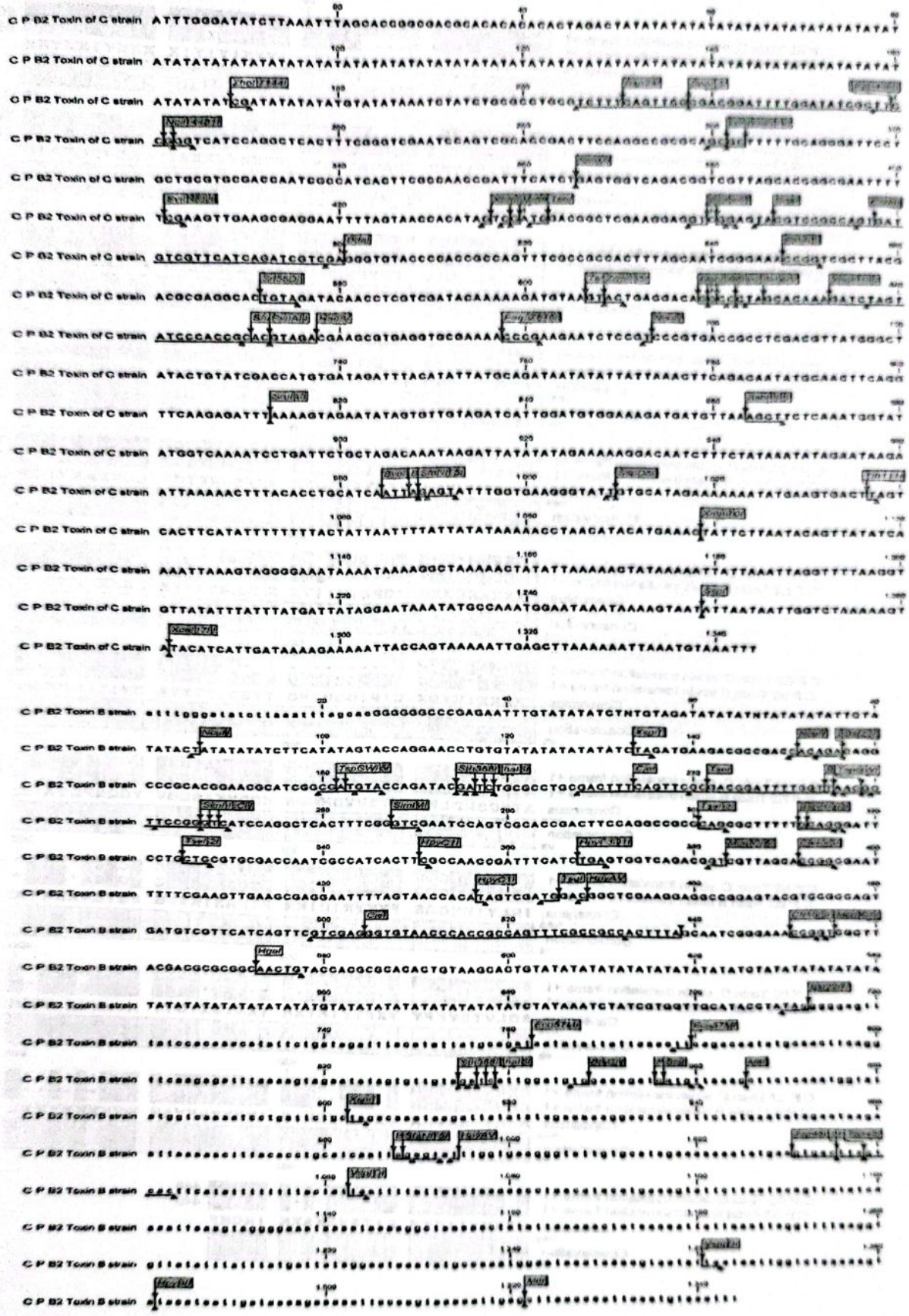


Fig (5) the restriction endonuclease analysis map of β_2 toxin gene of C. perfringens type B and C strains showing the enzymes that make a single cut.

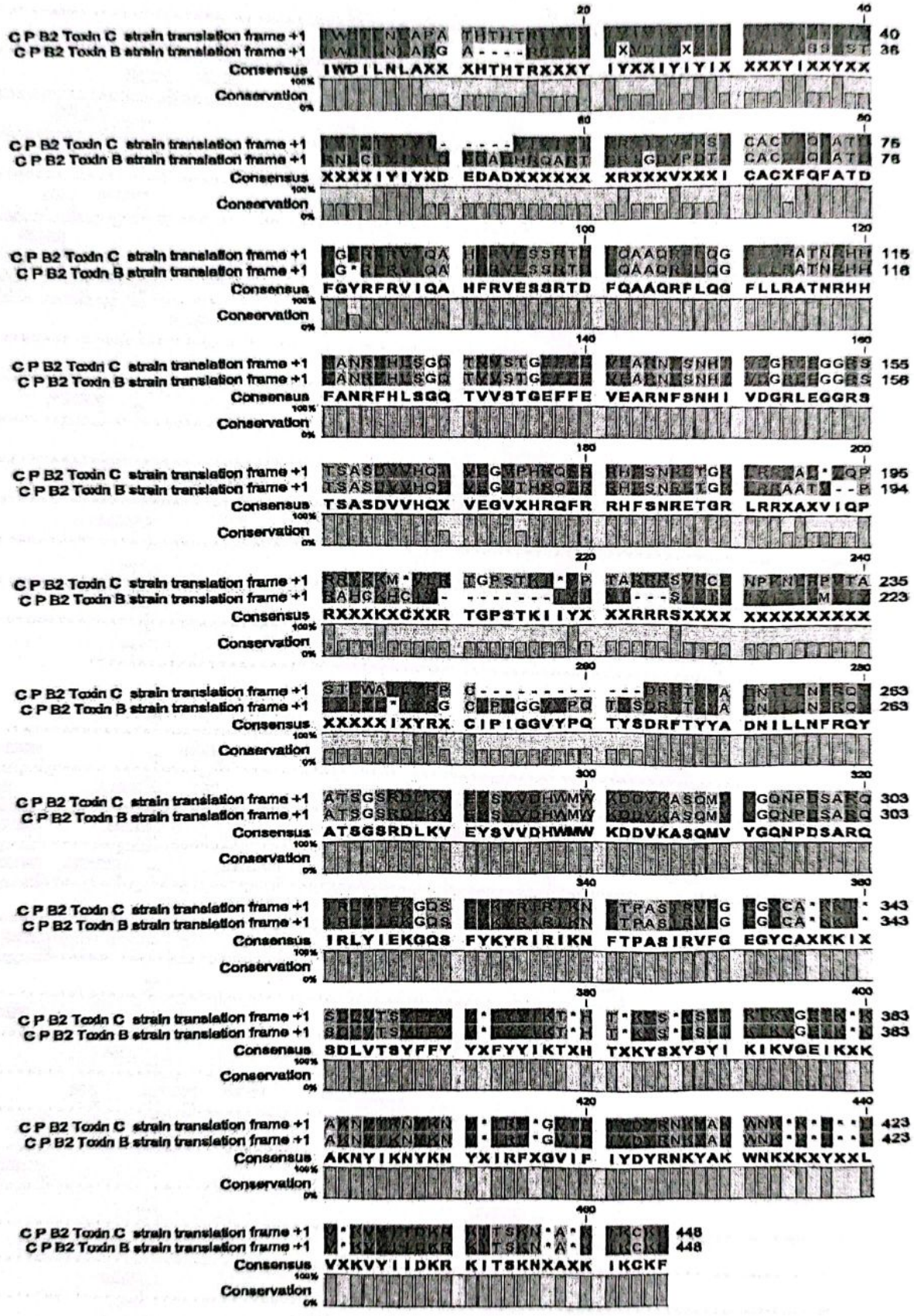


Fig (6) the alignment of the amino acid sequence of β_2 of C. perfringens type B and C strains

Nucleotide Frequencies	C P B2 Toxin C strain	C P B2 Toxin B strain
Adenine (A)	0,354	0,346
Cytosine (C)	0,164	0,162
Guanine (G)	0,179	0,182
Thymine (T)	0,303	0,308
Any nucleotide (N)	0,000	0,001
C + G	0,343	0,344
A + T	0,657	0,654

Nucleotide counts	C P B2 Toxin C strain	C P B2 Toxin B strain
Length	1344 nucleotides	1344 nucleotides
Adenine (A)	476	465
Cytosine (C)	220	218
Guanine (G)	241	245
Thymine (T)	407	414
Any nucleotide (N)	0	2
C + G	461	463
A + T	883	879

Table (1) the Nucleotide counts and frequencies of the β_2 toxin of C. perfringens type B and C strains

DISCUSSION

Clostridium perfringens has a single circular chromosome with a GC content ranging from 24 to 55%. C. perfringens has a relative low GC content comparing to that of the majority of Gram-positive bacteria. The chromosome contains 10 rRNA genes and 96 tRNA genes. The genome contains genes that encode a variety of transporters that transport "amino acids, cations/anions, carbohydrates, and nucleosides/nucleotides". Like many Mycoplasma bacteria and Bacillus subtilis, C. perfringens arrange their genes in a way such that their transcriptional process orients in the same direction as their replication direction (Shimizu et al., 2002)

Clostridium perfringens cpb_2 toxin is a 28 KDa toxin that encoded by 1344 bp chromosomal gene and it is implicated in necrotic enteritis in animals and humans, therefore a vaccine directed against this toxin will be needed. DNA vaccines against cpb_2 toxin of C. perfringens type B and C will be a suitable choice only if a minimum degree of heterogeneity between types B and C is detected. Thus this study aimed to investigate the genetic diversity of cpb_2

On cpb_2 amplification by PCR , several nonspecific bands were observed (data not shown) when annealing at temperature less than 60 °C so gradient annealing (50 – 60 °C) temp was first conducted. At a 60°C annealing temp, a band with 1350pb was observed with C. perfringens type B and C and another band with about 400pb was seen which indicated internal annealing of the primer within the gene this band was not eliminated even when the annealing temperature was elevated to 60°C. Higher annealing temp (over 60°C) results in no amplification even to the target sequence.

The cpb_2 gene was only amplified from the genomic DNA not plasmid DNA of C. perfringens type B and C standard strains that indicated the association of the gene only with the genomic DNA (photo 2), contrary to the findings of Waters et al., (2005) who recovered the cpb_2 gene from C. perfringens type A isolates from horses suffering from gastrointestinal diseases. Yet, he reported an extremely difference at the transcriptional level of the cpb_2 gene between pig CWC245 and horse isolates however he reported that the cpb2 upstream promoter region is identical in both pig and horse isolates. The 1373 bp sequenced fragment of cpb2 gene does not contain any features which would account for different cpb2 expression levels between pig strain CWC245 and horse isolates. Therefore, it can be envisioned that the relevant difference(s) might be present outside of this fragment and be involved in regulating cpb_2 expression (Gibert et al., 1997). In a report by Miyamoto et al., 2006 he found that the enterotoxin gene (cpe) is usually chromosomal in food poisoning isolates but plasmid-borne in C perfringens sporadic diarrhea (SPOR) and antibiotic-associated diarrhea (AAD) isolates. Previous studies determined that type A SPOR isolate F5603 has a plasmid (pCPF5603) carrying cpe, IS1151, and the cpb2, while type A SPOR isolate F4969 has a plasmid (pCPF4969) lacking cpb2 and IS1151 but carrying cpe and IS1470-like sequences. Additional PCR analyses, sequencing studies and pulsed field gel electrophoresis experiments determined that AAD/SD isolates carry cpb2 and cpe on the same plasmid when IS1151 sequences are present downstream of cpe, but cpb2 and cpe are located on different plasmids in AAD/SD isolates where IS1470-like sequences are present downstream of cpe (Fisher et al., 2005).

The genetic analysis revealed that the gene amplified from both strains have nearly the same GC content (table 1) with the most of the gene sequence alterations occur within the middle of the gene away from either the N terminal (signal sequence) or the C terminal of the gene and most of which are base substations. this type of mutation is the most common forms of point mutation which is the transition that exchanges a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine, (C ↔ T) Which is usually caused by nitrous_acid, base mis-pairing (Ellis et al., 2001) most of which are missense mutations which results in alteration of the amino acid sequence of the translated protein (fig 6).

The other form of mutation which seen in this study is the dilations mutation (Intercalary Deletion / Interstitial Deletion) - a deletion that occurs from the interior of a gene. Although this type of mutation is fatal for the gene transcription (as it may cases frameshift) yet the cpb_2 still functioning as the toxin can be recovered from the culture filtrate of the C. perfringens. (Data not shown) which denote that the genetic loss did not cause any frameshift and that this process may be controlled by other genetic element such as the IS1470-like sequences (Fisher et al., 2005).

While the current studies offer new insights into the sequences and diversity of the cpb_2 gene of B and C strains of C. perfringens, additional researches are needed to identify B and T cell epitope mapping of the protein and investigating there heterogeneity on immunological bases.

REFERENCES

Bacciarini, L. N., P. Boerlin, R. Straub, J. Frey, and A. Gro¨ne. (2003): Immunohistochemical localization of the Clostridium perfringens 2-toxin in the gastrointestinal tract of horses. Vet. Pathol. 40:376–381.

Bueschel, D. M., B. H. Jost, S. J. Billington, H. T. Trinh, and J. G. Songer. (2002):Prevalence of cbp2, encoding beta2 toxin, in Clostridium perfringens field isolates: correlation of genotype with phenotype. Vet. Microbiol. 94:121–129.

Ellis, A. N.; Ciocci, S. and German, J.: (2001): Back mutation can produce phenotype reversion in Bloom syndrome somatic cells Hum Genet. , 108, (2): 167–173

Fisher, D.J; Miyamoto, K.; Harrison ,B.; Akimoto, S., Sarker, M.R. and McClane, B.A. (2005):Association of beta2 toxin production with Clostridium perfringens type A human gastrointestinal disease isolates carrying a plasmid enterotoxin gene. Mol Microbiol. 56(3):747-62.

Garmory, H. S., N. Chanter, N. P. French, D. Bueschel, J. G. Songer, and R. W. Titball. (2000): Occurrence of Clostridium perfringens beta2-toxin amongst animals, determined using genotyping and subtyping PCR assays. Epidemiol. Infect. 124:61–67.

Gibert, M., C. Jolivet-Reynaud, and M. R. Popoff. (1997): Beta2 toxin, a novel toxin produced by Clostridium perfringens. Gene 203:65–73.

Helen Jost. B., Billington,S. J.; J Hien T. Trinh, Dawn M. Bueschel, and J. Glenn Songer (2005): Atypical cpb2 Genes, Encoding Beta2-Toxin in Clostridium perfringens Isolates of Nonporcine Origin infection and immunity, p. 652–656 Vol. 73, No. 1

Herholz, C., R. Miserez, J. Nicolet, J. Frey, M. Popoff, M. Gibert, H. Gerber, and R. Straub. (1999): Prevalence of beta2-toxigenic Clostridium perfringens in horses with intestinal disorders. J. Clin. Microbiol. 37:358–361.

Hunter, S. E., J. E. Brown, P. C. Oyston, J. Sakurai, and R. W. Titball. (1993): Molecular genetic analysis of beta-toxin

of Clostridium perfringens reveals sequence homology with alpha-toxin, gamma-toxin, and leukocidin of Staphylococcus aureus. Infect. Immun. 61:3958–3965.

James, G.; Francisco, A. and Bruce A. M. (2007): Identification of a Prepore Large-Complex Stage in the Mechanism of Action of Clostridium perfringens Enterotoxin Infect Immun. 75(5): 2381–2390.

Johnson, M. W., G. R. Fitzgerald, M. W. Welter, and C. J. Welter. (1992): The six most common pathogens responsible for diarrhea in newborn pigs. Vet. Med. 87:382–386.

Jolivet-Reynaud, C., M. R. Popoff, M. A. Vinit, P. Ravisse, H. Moreau, and J. E. Alouf. (1986):Enteropathogenicity of Clostridium perfringens beta toxin and

Lawrence, G. W., D. Lehmann, G. Anian, C. A. Coakley, G. Saleu, M. J. Barker, and M. W. Davis. (1990): Impact of active immunisation against enteritis necroticans in Papua New Guinea. Lancet 336:1165–1167.

Miyamoto, K.; Fisher, D.J; Li, J.; Sayeed, S.; Akimoto, S. and McClane, B.A. (2006): Complete sequencing and diversity analysis of the enterotoxin-encoding plasmids in Clostridium perfringens type A non-food-borne human gastrointestinal disease isolates.J Bacteriol.; 188 (4):1585-98.

Rodney K. Tweten (2001):Clostridium perfringens beta toxin and Clostridium septicum alpha toxin: their mechanisms and possible role in pathogenesisVet.Microbiol. 82 (3): 1-9

Shatursky, O.; Bayles, R.; Rogers, M.; Jost, H.; Songer, J. G. and Tweten, R . K. (2000): Clostridium perfringens Beta-Toxin Forms Potential-Dependent, Cation-Selective Channels in Lipid Bilayers Infect and Immu., 68,(10): 5546–5551

other clostridial toxins. Zentbl. Bakteriol. S15:145–151.

Jost, B. H., S. J. Billington, H. T. Trinh, D. M. Bueschel, and J. G. Songer. (2005): Atypical cpb2 genes, encoding beta2-toxin in Clostridium perfringens isolates of nonporcine origin. Infect. Immun. 73:652–656.

Jun Sakurait and Charles Duncant (1978): Some Properties of Beta-Toxin Produced by Clostridium perfringens Type C. Infect and Immu, 21, (2): 678-680

Klaasen, H. L., M. J. Molkenboer, J. Bakker, R. Miserez, H. Hani, J. Frey,M. R. Popoff, and J. F. van den Bosch. (1999): Detection of the beta2 toxin gene of Clostridium perfringens in diarrhoeic piglets in The Netherlands and Switzerland. FEMS Immunol. Med. Microbiol. 24:325–332.

Shimizu, T.; Ohtani, K.; Hirakawa, H.; Ohshima, K.; Yamashita, A.; Shiba, T.; Ogasawara, N.; Hattori, M.; Kuhara S.and Hayashi, H.: (2002): Complete genome sequence of Clostridium perfringens, an anaerobic flesh-eater Proc. Natl. Acad . Sci. U S A. 99 (2): 996-1001.

Steinthorsdottir, V., H. Halldorsson, and O. S. Andresson. (2000): Clostridium perfringens beta-toxin forms multimeric transmembrane pores in human endothelial Cells. Microb. Pathog. 28:45–50.

Songer, J. G. (1996):Clostridial enteric disease of domestic animals. Clin. Microbiol. Rev. 9: 216–234.

Taylor, D. J. (1984):Causes of enteritis in young piglets. Proc. Pig. Vet. Soc. 11:56–66.

Timoney, J. F., J. H. Gillespie, F. W. Scott, and J. E. Barlough. (1988): Hagan and Bruner's microbiology and infectious diseases of domestic animals.Comstock Publishing Associates, New York, N.Y.

Thiede, S., R. Goethe, and G. Amtsberg. (2001): Prevalence of beta2 toxin gene of

Clostridium perfringens type A from diarrhoeic dogs. Vet. Rec. 149:273– 274.

Waters, M., A. Savoie, H. S. Garmory, D. Bueschel, M. R. Popoff, J. G. Songer, R. W. Titball, B. A. McClane, and M. R. Sarker. (2003): Genotyping and phenotyping of beta2-toxigenic Clostridium perfringens fecal isolates associate with gastrointestinal diseases in piglets. J. Clin. Microbiol. 41:3584–3591.

Waters, M.; Raju, D.; Helen S. Garmory; Popoff,R. M. and Sarker,M.R (2005): Regulated Expression of the Beta2-Toxin Gene (cpb2) in Clostridium perfringens Type A Isolates from Horses with Gastrointestinal Diseases J. Clin. Microbiol, vol. 43, no. 8: 4002–4009

Zhou, H.; Gong, J.; Brisbin J.; Yu H.; Sarson A.J.; Si W. and Sharif S.
Transcriptional profiling analysis of host response to Clostridium perfringens infection in broilers
Poult Sci (2009): 88:1023-1032.

Summary

To study the argue of being present in both strains, The β2 toxin gene of C. perfringens B and C strains was amplified from extracted genomic and plasmid DNA by PCR and sequenced .the results declared the presence of the toxin gene cpb_2 in the genomic but not plasmid both DNA of both strains but with a great degree of heterogeneity on the bases of nucleotides sequence. The DNA sequencing revealed that the difference in the nucleotide sequence was found in the middle of the gene between nt 35 – 220 with the most diversity lying between nt 550 -700 downstream to 5' end of the gene. At nt 36 – 44, lies a pronounced divergence of the cpb_2 from B strain from the consensus sequence where C strain marked the divergence at nt 139– 150 which indicate a great diversity between B and C strains.

التحليل الجينى تصنيف جين سموم β2 لميكروب الكلستيريدم برفرنجينزنوعية B و C

يوسف عادل سليمان، محمد على مخاريطة
المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية - العباسية - القاهرة

لدراسة مدي تواجد جين β2 في عترتي البي و السي لميكروب C. perfringens *قامت هذه الدراسة علي تضخيم الجين الخاص بهذا السم باستخدام تفاعل البلمرة المتسلسل علي الحامض النووي والبلازميد المعزول من هذه العترات. و قد أفادت هذه الدراسة تواجد الجين الخاص بسم* β2 في الحامض النووي و ليس البلازميد المستخلص من كلتا العترتان و لكن بدرجة من الاختلافات. و قد وجد ان معظم هذه الاختلافات تقع في منتصف الجين بين القاعدة 35 – 220 و كذلك 550 – 700 . فالاختلاف الواقع عند القاعدة 36 – 44 في العترة بي يختلف كثيرا عن ذلك الموجود في العترة سي و الذي يحوي علي اختلاف بين القاعدة 139 – 150 . و هذا يؤكد التباين الواضح في التتابع الجين لسم β2 في العترتان