

Comparison of Molecular Characterization Methods for *Clostridium botulinum* type C/D

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Introduction: *Clostridium botulinum* is differentiated into four groups (I to IV), which form botulinum neurotoxins (BoNTs) of seven types (A to G). Although most avian cases are caused by type C, C/D mosaic type has been reported recently which also belongs to the group III. The gold standard method to determine the genetic correlation is pulsed-field gel electrophoresis (PFGE). However, both reproducible and highly discriminating method has been needed to find. In this study, randomly amplified polymorphic DNA assay (RAPD) and BoNT gene partial sequencing were compared for this purpose.

Materials and Methods: We isolated 13 *C. botulinum* from cecum or liver of poultry and wild birds with a typical botulism symptom. The isolates were characterized genetically by PFGE, RAPD PCR, and BoNT gene sequencing. PFGE and RAPD PCR were performed as previously described (Skarin *et al.*, 2010). We designed the primers for BoNT gene PCR to discriminate interrelationship among the isolates and found the optimal annealing temperature (48 – 60 °C). Each purified DNA from PCR product was sequenced and analysed by CLC Main Workbench ver. 6.

Results: PFGE results showed just smear bands except the lambda ladders. Although there are continuous failures in PFGE experiment, we obtained RAPD PCR bands for finishing the dendrogram. In that dendrogram, however, the isolates from different organs in one organism were decided as unrelated strains ($\leq 95\%$). Therefore it didn't have reliability. So we tried BoNT PCR and sequencing amplified region, and calculated the correlation distance. Other strains were exactly same in sequence, but Q621 and D77 strains differed in different base, respectively. Q587 strain was noticeably distant from others. Q587 strain grow actively even after several subcultures and is supposed to produce stronger botulinum neurotoxin because of distinctive sequence.

Conclusions: BoNT gene characterization is the most distinguishable among three methods. We recommend our novel method, BoNT gene partial sequencing to discriminate *C. botulinum* type C/D in genetic scope. Q587 is needed to be characterized precisely in toxin production mechanism through further study.

References

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Involvement of Src/ NOX Signaling Pathway in Autophagic Cell Death by *Vibrio vulnificus* VvhA

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Introduction: VvhA, one of virulent factors of *Vibrio (V.) vulnificus*, induces acute cell death via necrotic and apoptotic cell death. Autophagy plays an important role in cell death control, but the functional role of VvhA in autophagic cell death is not elucidated yet.

Materials and Methods: Cytotoxicity, ROS production, changes in lipid raft components, autophagy flux and lysosomal membrane permeabilization were analyzed in human intestinal epithelial Caco-2 cell treated with recombinant protein (r) VvhA.

Results: We found that rVvhA significantly increased LC3 puncta formation, autophagy flux, and lysosomal membrane permeabilization in promoting the apoptotic death of human intestinal epithelial Caco-2 cells. rVvhA induced rapid phosphorylation of non-receptor tyrosine kinase, c-Src. rVvhA stimulates the lipid raft clustering of Rac1 with NOX for ROS production which was inhibited by the pretreatment of lipid raft disruptor, methyl- β -cyclodextrin. The NOX-mediated bacterial signal induced by rVvhA increased phosphorylation of ERK and Beclin-1 expression that are required for autophagy initiation. Finally, rVvhA induced autophagy-dependent imbalance of the Bcl-2/Bax ratio, the release of mitochondrial cytochrome c, and caspase-3/9 activation during its promotion of apoptotic cell death.

Conclusions: These results demonstrate that *V. vulnificus* VvhA induces autophagic cell death via Src-dependent NOX signaling pathway.

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V. vulnificus VvpE Promotes Tight Junction Disruption and Intestinal Colonization

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Introduction: *Vibrio(V.) vulnificus*, VvpE, is an elastase responsible for host surface adherence and vascular permeability; however, the roles of VvpE in the pathogenesis of *V. vulnificus*(WT) are poorly understood. In the present study, we have investigated the role of VvpE in regulation of intestinal tight junctions and the colonization of WT.

Materials and Methods: *In vivo* role of VvpE in tight junction disruption and intestinal colonization was assessed by the mutation and complementation of the *vvpE* gene from *V. vulnificus* in mouse infection models. Gain- and loss-of- function approaches were used to identify enterotoxicity and pro-inflammatory response in mice ileum tissue.

Results: We found that *vvpE* mutant prevents intestinal tight junction dysregulation due to a WT infection and maintains the physiological level of the epithelial paracellular permeability. Interestingly, the *vvpE* mutant exhibited defective intestinal colonization abilities, whereas WT colonization was significantly elevated in the ileum. Finally, the *vvpE* mutant negated the enterotoxicity, the breakdown of red blood cells, and pro-inflammatory responses, all of which are induced by the WT infection. In addition, we found that VvpE contributes to WT pathogenesis in multiple ways by interacting with intestinal proteins, including β -globin, Annexin A2, Annexin A4, F-actin, and integrin-1b.

Conclusions: These results demonstrate that VvpE plays an important role in promoting the tight junction disruption and intestinal colonization of *V. vulnificus*.

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P-012

Studies on pathogenesis of avian influenza viruses in mice by infection route

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Introduction: Highly pathogenic avian influenza virus (HPAI), H5N1 has been regarded as a strong pandemic candidate with high pathogenesis and mortality threatening the health of the human. Respiratory complications are general symptoms, but gastrointestinal symptoms have often been reported in human infected with HPAI H5N1.

Materials and Methods: Here we investigated whether infection route can affect the different types of pathogenesis with a mouse infection model. Mice were infected with avian influenza virus bearing three different degrees of subtypes (HPAI, low pathogenic avian influenza (LPAI), and seasonal influenza) by intranasal or oral route. The mice infected with HPAI by intranasal route showed higher mortality and viral infection in lung than those infected with seasonal influenza or LPAI.

Results: The virus was not detected in intestinal in all mice infected through intranasal route. However evident pathogenesis was observed in intestine of the mice infected with A/Vietnam/1194/2001 orally. Moreover, the virus was recovered in intestine as a 2.0 TCID₅₀/ml viral titer.

Conclusions: This mice study supported that some orally HPAI infection might cause enteropathogenesis like A/Vietnam/1194/2001 (H5N1) strain. It means that high dose infection of HPAI through nasal route or intake HPAI contaminated poultry can cause gastrointestinal infection and spread whole body including lung and respiratory tract.

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P-013

Sequence Analysis of Nucleocapsid Gene of Porcine Epidemic Diarrhea Virus isolated in 2015

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Introduction: Porcine epidemic diarrhea virus (PEDV), a porcine enteropathogenic coronavirus, causes lethal watery diarrhea in piglets resulting in large economic losses due to high mortality [4]. Morbidity and mortality in infected neonatal piglets less than 5 days old approach 100% because