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Comparison of Molecular Characterization Methods for *Clostridiumbotulinum* type C/D

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Introduction: *Clostridium. botulinum* is differentiated into four groups (I to IV), which form botulinumneurotoxins (BoNTs) of seven types (A to G). Although most avian cases arecaused by type C, C/D mosaic type has been reported recently which also belongsto the group III. The gold standard method todetermine the genetic correlation is pulsed-field gel electrophoresis (PFGE). However,both reproducible and highly discriminating method has been needed to find. Inthis study, randomly amplified polymorphic DNA assay (RAPD) and BoNT genepartial 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 typicalbotulism symptom. The isolates were characterized genetically by PFGE, RAPD PCR, and BoNT gene sequencing. PFGE and RAPD PCR were performed as previouslydescribed (Skarin *et al.,* 2010). We designedthe primers for BoNT gene PCR to discriminate interrelationship among theisolates and found the optimal annealing temperature (48 – 60℃). Each purified DNA from PCR product was sequencedand analysed by CLC Main Workbench ver. 6.

Results: PFGE results showed just smearbands except the lambda ladders. Although there are continuous failures in PFGEexperiment, we obtained RAPD PCR bands for finishing the dendrogram. In thatdendrogram, however, the isolates from different organs in one organism weredecided as unrelated strains (≤95%). Therefore it didn't have reliability. So we triedBoNT PCR and sequencing amplified region, and calculated the correlationdistance. Other stains were exactly same in sequence, but Q621 and D77 strainsdiffered in different base, respectively. Q587 strain was noticeably distantfrom others. Q587 strain grow actively even after several subcultures and issupposed to produce stronger botulinum neurotoxin because of distinctivesequence.

Conclusions: BoNT gene characterization isthe 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 becharacterized precisely in toxin production mechanism through further study.

References

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P-010

Involvement of Src/ NOX Signaling Pathway in Autophagic CellDeath by Vibrio vulnificus VvhA

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Introduction: VvhA, one of virulent factorof V*ibrio (V.) vulnificus*, induces acute cell death via necrotic and apoptoticcell death. Autophagy plays important role in cell death control, but thefunctional role of VvhA in autophagic cell death is not elucidated yet.

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

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

Conclusions: These resultsdemonstrate that V. vulnificus VvhA induces autophagic cell death viaSrc-dependent NOX signaling pathway.

References

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V.vulnificus VvpE Promotes Tight Junction Disruption and Intestinal Colonization

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