

DOCTORAL THESIS

Applications of 16S rRNA metagenomics and metabolomics in correlation of toxicity of puffer fishes with gut microbiota and identification of potential precursors in tetrodotoxin biosynthesis

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ABSTRACT

Tetrodotoxin (TTX) is a lethal neurotoxin isolated mainly from the organs of wild puffer fishes. Although the neurotoxicity mechanisms of TTX are well known, the TTX origin and the biosynthetic mechanisms inside its hosts remain unresolved. In recent decades, the numerous reports of TTX-producing bacteria strongly suggested its bacterial origin. However, this origin is currently being challenged by the low and inconsistent TTX productions *in vitro* by the previously reported TTX-producing bacteria. Culturable TTX-producing bacteria were frequently isolated and reported from the guts of TTX-bearing animals including puffer fishes, however, these bacteria were estimated to account for 0.1% of the total gut bacteria. Moreover, the identification and functions of the non-culturable gut bacteria participating in TTX biosynthesis have never been reported. I hypothesize that the puffer fish gut bacteria and the entire gut environment serve as a functional integrality responsible for TTX biosynthesis. In this study, 16S rRNA amplicon metagenomics pipeline was established to profile the entire gut bacterial structures of both toxic and non-toxic puffer fishes respectively. UniFrac based principal coordinate analysis showed that bacterial diversities were significantly different (P -value < 0.001) between the gut environments of toxic puffer fishes and the non-toxics. *Vibrio* and Cyanobacteria were identified as centralities of gut bacteria co-occurrence network in toxic puffer fishes, implying their key roles in TTX biosynthesis. The results of metagenome prediction and gene set enrichment indicated that arginine biosynthesis was significantly enriched (P -value < 0.05) in the toxic group. To further investigate the roles of key bacteria and arginine biosynthesis in producing TTX, metabolomics pipeline was established along with 16S rRNA amplicon metagenomics to monitor the dynamics of metabolites and bacterial compositions in guts of toxic puffer fishes during their detoxification process. The average TTX concentrations in the liver after a 60-day culture ($6.41 \pm 3.00 \mu\text{g/g}$) was found significantly lower (P -value < 0.01) than that of the same species from the wild ($31.86 \pm 22.20 \mu\text{g/g}$). The relative abundance of *Vibrio* was found positively correlated with the liver TTX concentrations. With the increase of culture periods, the relative abundance of *Vibrio* and Cyanobacteria decreased. In addition, both the metabolites and functional genes in arginine biosynthesis metabolic pathway were found significantly down-regulated (P -value < 0.05). These results indicated that both *Vibrio* and Cyanobacteria bacterial symbionts participated in TTX biosynthesis using arginine as a potential precursor in the gut environment of toxic puffer fishes.

Keywords: Tetrodotoxin; Puffer fishes; 16S rRNA amplicon metagenomics; Metabolomics

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