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Exploring the Microbiome of *Callinectes sapidus* (Maryland Blue Crab)

Padmini Ramachandran,[a] Elizabeth Reed,[a] Andrea Ottesen[a]

[a]Division of Microbiology, Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, Maryland, USA

ABSTRACT The Maryland blue crab (*Callinectes sapidus*) is a treasured food, especially in areas surrounding the Chesapeake Bay. It has huge economic impact on commerce, and thus, understanding the bacterial, fungal, and viral constituents of its microbiome provides valuable information to safely manage aquaculture, handling, and cooking of this valuable commodity.

The Atlantic blue crab, commonly referred to as the Maryland blue crab (*Callinectes sapidus*), is a revered part of the diets of Marylanders and non-Marylanders alike. Describing the microbiome of this popular species provides valuable information to better understand the health risks for crab aquaculture and risks to consumers from handling or consuming inadequately cooked parts (1, 2). To date, one study has described the culture-independent anatomical microbial composition of the *Callinectes sapidus* carapace (chitinous "shell"), gut, and hemolymph microbiota using amplicons of 16S rRNA genes (3). A core community of *Proteobacteria* was described for all of these parts. Previous culture-based work identified pathogens, such as *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* associated with gills, viscera (intestines), meat of healthy crabs, and hemolymph of diseased crabs in commercial tanks. The relative abundances of pathogens varied significantly among diverse crab parts. The shotgun metagenomic data presented here provide culture-independent and PCR-bias-free insight into the native microbiome and distinct groupings of microbial taxa associated with different crab parts. Delineation of the crab parts used for this study was approached from an eater's perspective; legs were grouped together, claws were grouped together, and meat was separated (as cleanly as possible) from intestines and other internal organs. Crab parts were homogenized, and DNA was extracted using the Qiagen DNeasy blood and tissue kit and stored at −20°C for subsequent Nextera XT library preparation (Illumina, San Diego, CA). Additionally, for culture-dependent description, crab parts were incubated at 37°C in modified buffered peptone water (mBPW) broth for 24 h, and DNA was prepared as described above. Libraries were sequenced on an Illumina NextSeq 550, and data were analyzed using CosmosID bioinformatic pipelines. The data supported previous work that described a core proteobacterial community across all crab parts, in this case, composed of *Vibrio*, *Shewanella*, *Ralstonia*, and *Pseudoalteromonas* species. Certain taxa were shared and/or unique to different crab parts, such as *Alivibrio* spp., which were associated only with the gut, and the absence of *Exiguobacterium* spp., which were unique to the gut. *Citrobacter* spp. were unique to crab meat. Surprisingly, crab claws and meat were much more diverse than the gut, hosting *Psychrobacter*, *Propionibacterium*, *Shewanella*, *Exiguobacterium*, *Providencia*, *Ralstonia*, *Proteus*, *Clostridium*, *Pseudoalteromonas*, *Lysinibacillus*, *Enterococcus*, and *Vibrio* species. Uncultured crabs were dominated by *Psychrobacter* and *Propionibacterium* spp., while cultured crab parts were dominated by *Shewanella*, *Exiguobacterium*, and *Vibrio* species. The cultured crab microbiota also supported the growth of *Exiguobacterium*, *Lysinibacillus*, *Shewanella*, and *Enterococcus*

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Address correspondence to Padmini Ramachandran, padmini.ramachandran@fda.hhs.gov.

species. The incidence of *Vibrio* species was significant in all cultured parts except for claws. Phage elements also provided interesting contrasting signatures between cultured and uncultured crab parts. Uncultured crabs were dominated by *Psychrobacter* phages and *Hop trefoil cryptic virus*, and cultured crabs were dominated by *Lactococcus*, *Vibrio*, and *Enterobacteria* phages.

Accession number(s). All data have been deposited in the Sequence Read Archive under accession numbers SRR6938014 to SRR6938036 under the MetagenomeTrakr umbrella (BioProject number PRJNA448684).

REFERENCES

1. Davis JW, Sizemore RK. 1982. Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas. Appl Environ Microbiol 43:1092–1097.
2. Sizemore RK, Colwell RR, Tubiash HS, Lovelace TE. 1975. Bacterial flora of the hemolymph of the blue crab, *Callinectes sapidus*: numerical taxonomy. Appl Microbiol 29:393–399.
3. Givens CE. 2012. A fish tale: comparison of the gut microbiome of 15 fish species and the influence of diet and temperature on its composition. Ph.D. thesis. University of Georgia, Athens, GA.