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Analysis of the bacterial microbiome in the small octopus, *Octopus variabilis*, from South Korea to detect the potential risk of foodborne illness and to improve product management

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ABSTRACT

The small octopus (*Octopus variabilis*) is a popular seafood in many countries including South Korea. Because it is often consumed uncooked, the microorganisms in it often cause food poisoning. Therefore, analyzing the microbiome of the small octopus can help to understand the risk of food poisoning and manage octopus products better. A total of 40 small octopuses were collected from four sites in November and August. The microbiota was analyzed using Illumina Miseq sequencing, and the amount of bacteria was quantified by real-time PCR. In addition, we analyzed the influence of *Vibrio vulnificus* infection on the microbiome of the small octopus through artificial infection experiments. *Bacteroidetes* was the predominant phylum in August, and *Proteobacteria* was predominant in November. The composition of the microbiota in octopus depended on sampling region and season. The potential risk of foodborne illness from small octopus consumption might be higher in August than in November due to the abundance of potential pathogens. In the infection experiment, the proportion of *V. vulnificus* increased only at 27 °C. The composition and functional gene profiles of the microbiota varied in a similar manner between non-infected and infected samples over time at the same temperature. These results indicated that the indigenous microbiota in small octopus could inhibit colonization by *V. vulnificus* during storage. Although further studies are necessary to clarify these results, our results could help us better understand food poisoning through octopus ingestion and manage products.

1. Introduction

The small octopus (*Octopus variabilis*) is one of the most popular seafood as healthy food in Asian countries. Octopus mainly comprises water and protein and has low fat content and high proportions of polyunsaturated fatty acids (Vaz-Pires & Barbosa, 2004). Fishing rates have increased in recent times due to the increased consumption of octopus (Boyle & Rodhouse, 2005). Because of their habits of filter-feeding and inhabiting tidal flat sediments, small octopus accumulate rich and diverse microbiota (Prieur, Mével, Nicolas, Plusquellec, & Vigneulle, 1990). The small octopus was reported to be in the top five of the 50 most potentially hazardous foods consumed in South Korea (Park et al., 2013). Raw small octopus is generally eaten in Korean restaurants, and this can cause food poisoning through foodborne pathogens. The microbiota of small octopus includes indigenous bacteria as well as transient or non-indigenous bacteria, including potentially pathogenic microorganisms, can be causal agents of food poisoning (Berg, 1996; Moriarty, 1997). The composition of the microbiota is affected by various environmental conditions of the habitat; it can also vary during storage and delivery to consumers. However, there is very little data on microbiota in small octopus. When the raw octopus is consumed, the whole body is digested; therefore, investigating the entire microbiota of the small octopus is important for understanding the risk of food poisoning and managing the products.

Vibrio spp. have been reported to be the major bacterial agents of foodborne illnesses in Korea and Japan (Lee, Lee, Kim, & Park, 2001). They inhabit from the deep sea to shallow aquatic environments, and are predicted to be important etiological agents of diseases in the coming years, due to ocean warming (Paillard, Le Roux, & Borrego, 2004; Reen, Almagro-Moreno, Ussery, & Boyd, 2006). Pathogenic bacteria of the *Vibrio* spp. have been isolated from seafood worldwide and include several important pathogens of aquatic organisms (DaSilva et al., 2012). Among them, *V. vulnificus* has been the focus of research in the last decade because of its pathogenicity to both humans and fish; therefore, improved methods for its detection in small octopuses are available (Arias, Macian, Aznar, Garay, & Pujalte, 1999; Lee,

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Eun, & Choi, 1997). *Vibrio* can directly colonize octopus or contaminate them from other seafood during processing or storage in the market. *Vibrio* can influence the microbiota of the small octopus by interacting with the indigenous microbes. However, there is very little data on these issues, including uncultured bacteria. Therefore, it is necessary to analyze the effects of *Vibrio* infection on indigenous microbiota in small octopus before consumption.

In this study, we investigated the microbiota of small octopuses, collected from different sampling sites in August and November, using the MiSeq system with the 16S rRNA genes. We then compared the differences of microbiota, based on the regions and the sampling times. In addition, we assessed the influence of pathogens on the composition and potential functions of the indigenous microbiota through artificial infection experiments. Results in this study can extend our understanding of food poisoning by small octopus, and help to manage products according to information of microbiome.

2. Materials and methods

2.1. Sample collection

A total of 40 small octopus samples were collected from four different sites in November 2015 (relatively low temperature of seawater; 16.7 ± 0.7 °C) and August 2016 (relatively high temperature of seawater; 25.7 ± 2.2 °C) (Fig. 1). The sampling sites were chosen based on the areas with maximum production in the western and southern coasts of South Korea, and the sampling months were chosen based on the season with maximum consumption (KOSIS, 2017). Five small octopuses were randomly selected at each site, and transported in an icebox to the laboratory. Bacterial cells were detached from whole octopus using a spindle (microorganism homogenizer, Korea patent registration: 10-2010-0034930). Rotation and vibration were conducted by a direct drive motor in a stomach bag in the spindle. The bacterial cells obtained from each sample were diluted in 255 mL of buffered peptone water (10 g peptone, 5 g sodium chloride, 3.5 g disodium phosphate, 1.5 g potassium dihydrogen phosphate with pH 7.2), and stored at − 80 °C before metagenomic DNA extraction.

2.2. Artificial infection of Vibrio vulnificus on small octopus

The influence of *V. vulnificus* infection on the indigenous microbiota of small octopuses during storage was analyzed by artificially infecting octopuses with *V. vulnificus* and stored in sterile containers at 27 °C (room temperature) and 4 °C. A total of 32 small octopus samples obtained from site A in November were used to artificial infection experiments based on consumption amount (KOSIS, 2017). *V. vulnificus* FORC_037 was cultivated at 30 °C in a Luria-Bertani medium (LB; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 2% NaCl for 4 h until an optical density at 600 nm (OD_{600}) of 2.5 was achieved. The number of infected cells was 1.0×10^6 cells/g, which was the reported infective dose of *Vibrio* spp. (Huss, Ababouch, & Gram, 2003; Jeong & Satchell, 2012). *V. vulnificus* was evenly sprayed on the small octopuses. The temporal shifts of the microbiota were investigated after 1 h, 4 h, and 12 h. Since small octopuses tended to decompose after 12 h of storage at 27 °C, we analyzed the microbiota until 12 h of storage to compare between different temperatures. The shifts in the microbiota of uninfected samples during the same storage period were also investigated as the control. These infection experiments were performed in duplicate. Bacterial cells on the small octopuses were detached as described earlier, and the obtained cells were stored at − 80 °C before DNA extraction.

2.3. Metagenomic DNA extraction and quantitative real-time polymerase chain reaction

Metagenomic DNA was extracted from each sample using the phenol DNA extraction method as described in previous studies (Lee, Lee, Chung, Choi, & Kim, 2016; Naravaneni & Jamil, 2005). Briefly,

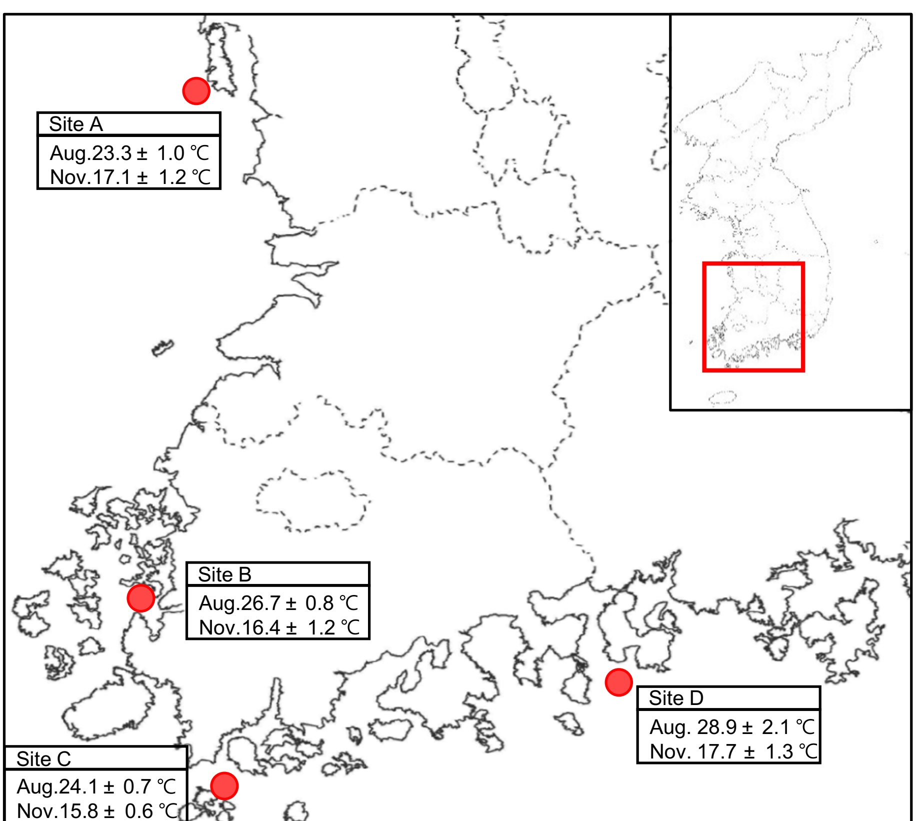

Fig. 1. Sampling sites of small octopuses. Samples were obtained from four different sites in November 2015 and August 2016. Each site was chosen based on the production of small octopuses in South Korea. The water temperature at the sampling time is presented below each sampling site.