Survivals of *Vibrio parahaemolyticus* and *Escherichia coli* in a Gastropod Mollusc, *Heminerita japonica*

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ABSTRACT. *Vibrio parahaemolyticus* strains D-3 and R-13 were found to be cleared within 7 days from a marine neritid gastropod mollusc, *Heminerita japonica*, maintained in artificial seawater with salinities of 15, 25 and 35 permil (‰) at 25°C. *Escherichia coli* strain YS-2 survived at a level of 10² colony forming units per gram in the mollusc maintained in 15‰ water for up to 14 days and fell to non-detectable level within 7 days in a 35‰ salinity group. The ability of *H. japonica* to clear these organisms seems to be less active than that of a marine species, *Nerita albicilla*, and more active than that of an estuarine species, *Clithon retropictus*.—**KEY WORDS:** gastropod mollusc, *Heminerita japonica*, salinity, survival, *Vibrio parahaemolyticus*.

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Vibrio parahaemolyticus is an estuarine organism which can cause gastroenteritis in human after consumption of contaminated seafoods. The organism was detected at high levels from various specimens at Hashizu estuary, Japan, in which a neritid gastropod mollusc, *Clithon retropictus*, was found to be an important reservoir of Kanagawa-positive strains [4]. The organism survived in the digestive tracts of the gastropod maintained in UV-irradiated artificial seawater for at least 21 days after exposure to contaminated water but was cleared from a marine neritid, *Nerita albicilla*, within 3 days [7, 8]. However, the mechanism for *C. retropictus* to retain *V. parahaemolyticus* is not yet elucidated sufficiently.

The salinity levels of water at the molluscan habitats are 1–20 permil (‰) for *C. retropictus* and 30–35‰ for *N. albicilla*, so that the variation of the defense system among neritid molluscs was suspected to be related with salinity preferences of the molluscs. *Heminerita japonica* is a neritid mollusc inhabiting littoral zones of the rocky coasts of southern Japan with the highest density at salinity levels of 20–30‰, higher than those of *C. retropictus* and lower than those of *N. albicilla*. Therefore, survivals of *V. parahaemolyticus* and *Escherichia coli* in *H. japonica* were analyzed to compare the salinity dependency of the bacterial survivals in the mollusc with those in *C. retropictus* and *N. albicilla* in the present study.

MATERIALS AND METHODS

H. japonica, 0.5 to 1.0 g weight, collected at Cape Muroto in Japan was maintained in aquaria with 28l of UV-irradiated artificial seawater [8] with a salinity of 25‰ at 25°C for one week before examinations. Cultured diatom alga, *Navicula* strain F-1, adhered to plastic plates was given to the molluscs for continuous feeding throughout the study. The mollusc was confirmed non-detectable for *V. parahaemolyticus* and *E. coli* before examinations.

V. parahaemolyticus strains D-3 (Kanagawa-positive; nalidixic acid-resistant) and R-13 (Kanagawa-negative; rifampicin-resistant) and *E. coli* strain YS-2 (nalidixic acid-resistant) were used in this study, which were characterized in our previous paper [5, 8]. *V. parahaemolyticus* strains were grown in Trypticase Soy Broth (Difco) containing 1% NaCl at 37°C for 8 hr with shaking. The cells were washed with 25% artificial seawater and suspended at approximately 10⁶ viable units per ml in 25% artificial seawater. *E. coli* strain YS-2 was grown in Trypticase Soy Broth containing 0.5% NaCl and suspended as *V. parahaemolyticus* strains.

Ninety molluscs were incubated in 100 ml of the bacterial suspensions in 25‰ artificial seawater at 25°C for 24 hr with aeration, washed with distilled water and maintained in 15, 25 and 35‰ seawater in the UV-irradiated circulation system. Every 5 molluscs were sacrificed at appropriate time intervals to obtain homogenates of digestive tracts and digestive

glands in 35‰ artificial seawater supplemented with 0.1% bovine serum albumin (Sigma) using Polytron 10ST (Kinematica). Viable units of *V. parahaemolyticus* strains in the homogenates were estimated by plating on Bromothymol Blue-Lactose Agar (Nissui) plates supplemented with 5% NaCl and 5 µg/ml nalidixic acid or rifampicin after incubation at 37°C for 18 hr [8] and expressed as the number of colony forming units per gram (cfu/g) of the homogenates. Those of *E. coli* strain YS-2 were estimated on the Bromothymol Blue-Lactose agar plates supplemented with 50 µg/ml nalidixic acid. The experiments were repeated 3 times.

RESULTS

H. japonica ingested *V. parahaemolyticus* or *E. coli* strains was maintained in artificial seawater with salinities of 15, 25 and 35‰ at 25°C for up to 14 days. *V. parahaemolyticus* strains D-3 and R-13 fell to non-detectable level within 4 days in molluscs maintained in 25 and 35‰ artificial seawater and within 7 days in a 15‰ salinity group after exposure to contaminated water (Figs. 1 and 2).

E. coli strain YS-2 was detected at a level of 10^2 cfu/g for at least 14 days after exposure to contaminated water from the mollusc maintained in 15‰ artificial seawater and fell to 2.7 cfu/g at the 14th day in a 25‰ salinity group and to non-detectable level within 7 days in a 35‰ salinity group (Fig. 3).

DISCUSSION

C. retropictus, but not a marine gastropod, *Monodonta labio*, was found to be an important reservoir of Kanagawa-positive strains of *V. parahaemolyticus* [4]. *V. parahaemolyticus* and *E. coli* strains were found to survive in *C. retropictus* for at least 21 days after exposure to contaminated water but to be cleared from a marine neritid, *N. albicilla*, within 3 days [7,8]. *C. retropictus* hemocytes were found to be poor in migratory responses against *V. parahaemolyticus* and *E. coli* strains in artificial seawater, while *N. albicilla* hemocytes were active in the responses [6]. From these evidences, an ability of *C. retropictus* to retain *V. parahaemolyticus* and the poor-responses of hemocytes to the organism were supposed to be its strategy to survive in the fluctuating salinity environments. *N. albicilla* inhabiting rather stable salinity environments would have developed the normal defense system with

Fig. 1. Survival of *V. parahaemolyticus* strain D-3 in *H. japonica* maintained in aquaria filled with artificial seawater at 35(●), 25(○) and 15(■)‰ salinities.

Fig. 2. Survival of *V. parahaemolyticus* strain R-13 in *H. japonica* maintained in aquaria filled with artificial seawater at 35(●), 25(○) and 15(■)‰ salinities.

Fig. 3. Survival of *E. coli* strain YS-2 in *H. japonica* maintained in aquaria filled with artificial seawater at 35(●), 25(○) and 15(■)‰ salinities.

active hemocytes.

The ability of contaminated marine bivalve molluscs to clear coliform bacteria when placed in clean water for 48 hr is well known and has been exploited in artificial depuration systems. Marine bivalves

including *Crassostrea virginica* [1, 3] and *Mercenaria mercenaria* [1, 2] were shown to take longer times to clear vibrios, *V. vulnificus* and *V. harveyi*, than other Enterobacteriaceae. In our previous study [5], an estuarine bivalve, *Corbicula japonica*, retained *V. parahaemolyticus* at levels of 10^2 to 10^3 cfu/g for at least 28 days and cleared *E. coli* within 21 days after exposure to contaminated water. These bivalves might have developed the weak-responsibility to the vibrios in their long-term interactions.

H. japonica was observed to support survival of *E. coli* YS-2 and to clear two *V. parahaemolyticus* strains in the present study. Our previous observations that the *V. parahaemolyticus* and *E. coli* strains survived in *C. retropictus* and were cleared from *N. albicilla* led us to a hypothesis that the estuarine gastropod would have developed a commensalism with *V. parahaemolyticus* and *E. coli* in the pollutions of these organisms at the estuarine areas[8]. However, the hypothesis seems to be difficult to explain the responses of *H. japonica* to these organisms because *H. japonica* inhabiting littoral zones of the rocky coasts have an ability to retain *E. coli*. Neritid gastropods are supposed to have developed the peculiar non-responsibility to some organisms in the process of adaptation to fluctuating salinity in the estuarine environments. The strategy of the gastropods to adapt the defense system to the estuarine environments might be different from that of bivalves.

Rowse and Fleet [9] supposed that the Sydney rock oyster, *Crassostrea commercialis*, would suffer stress in low salinity conditions from the observations that the oyster cleared *Salmonella charity* and *E. coli* rapidly in 32 and 47‰ seawater and slowly in 15 and 20‰ seawater. In our previous studies [7, 8], however, *V. parahaemolyticus* survived at high levels in *C. retropictus* and was cleared from *N. albicilla* in salinity levels of water at the habitats of

each mollusc. In the present study, *H. japonica* cleared *V. parahaemolyticus* within 4 days and retained *E. coli* for at least 14 days in salinity conditions of the molluscan habitat. From these evidences, three typical species of neritid molluscs with different salinity preferences were confirmed to take different behavior for the survival of *V. parahaemolyticus* related with salinity conditions of water.

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