Source dependent virulence of *Vibrio harveyi* against cultivable shrimps

Shrimp culture in India is currently facing several hurdles including the luminous bacterial disease in the hatcheries. The bacterial species *Vibrio harveyi* has been reported as the causative agent of the disease in shrimp hatcheries of various countries[1–5], including India[6]. However, differences in the virulence pattern between the isolates of *V. harveyi* obtained from water and shrimps have not been reported. In the present communication, different levels of virulence in *V. harveyi* isolated from the sea water off Mangalore and reisolated strain from the haemolymph of experimentally-infected shrimp are reported.

Table 1. Cumulative percentage mortality of juvenile *P. indicus* and *P. monodon* injected intramuscularly with different doses of *V. harveyi* strain obtained from sea water

Test animal	Duration (h)	Control	Cumulative percentage mortality: Bacterial dose (cfu/shrimp) 10	10^2	10^3	10^4	10^5	10^6
P. indicus	12	–	–	–	5	15	20	35
P. monodon		–	10	10	20	30	40	50
P. indicus	24	–	–	5	5	25	30	45
P. monodon		–	15	20	30	45	65	80
P. indicus	36	–	5	10	10	30	40	60
P. monodon		–	20	30	45	65	90	100
P. indicus	48	–	5	20	25	50	55	80
P. monodon		–	30	40	60	80	100	–
P. indicus	60	–	5	20	35	65	80	100
P. monodon		–	35	50	75	90	–	–
P. indicus	72	–	10	20	50	70	95	–
P. monodon		–	40	65	85	100	–	–
P. indicus	84	–	15	30	65	90	100	–
P. monodon		–	40	70	95	–	–	–
P. indicus	96	–	20	35	70	100	–	–
P. monodon		–	45	75	100	–	–	–

Table 2. Cumulative percentage mortality of juvenile *P. indicus* and *P. monodon* injected intramuscularly with different doses of *V. harveyi* strain obtained from haemolymph

Test animal	Duration (h)	Control	Cumulative percentage mortality: Bacterial dose (cfu/shrimp) 10	10^2	10^3	10^4	10^5
P. indicus	12	–	10	10	20	30	40
P. monodon		–	10	25	45	85	100
P. indicus	24	–	10	20	30	45	60
P. monodon		–	20	35	30	50	–
P. indicus	36	–	20	30	45	65	90
P. monodon		–	20	40	65	100	–
P. indicus	48	–	30	45	60	80	100
P. monodon		–	35	80	100	–	–
P. indicus	60	–	35	50	75	90	–
P. monodon		–	50	100	–	–	–
P. indicus	72	–	40	60	85	100	–
P. monodon		–	55	–	–	–	–
P. indicus	84	–	40	70	100	–	–
P. monodon		–	60	–	–	–	–
P. indicus	96	–	45	75	–	–	–
P. monodon		–	70	–	–	–	–

The larvae of *P. indicus* and *P. monodon* (PL 10) obtained from hatcheries (Deejay Hatcheries Pvt. Ltd., Honnavar and Sumasri Hatcheries, Kulai) were reared in the laboratory in sea water recirculatory system up to juveniles. The size of the juveniles of *P. indicus* ranged between 4.9 cm and 5.4 cm, while the size of the juveniles of *P. monodon* ranged from 5.2 cm to 5.8 cm. For isolation of luminous bacteria from water and shrimp, SWC agar medium with 3 ml of glycerol per litre was used[7].

Sea water was collected from the Arabian Sea off Mangalore using sterile water sampler. Sea water was aseptically transferred to sterile 20 ml McCartney bottles and brought to the laboratory and 0.1 ml of water was inoculated on precooled SWC agar plates incubated at room temperature (28 ± 2°C). After 36 h of incubation, the luminous colonies were randomly picked up in dark using sterile toothpicks. For isolation of *V. harveyi* from haemolymph of shrimp (*P. indicus*), the animals were first exposed to *V. harveyi* through immersion and the bacteria were re-isolated after 16 h of exposure. The isolates of *V. harveyi* obtained from sea water and reisolated from haemolymph were used for virulence study challenging against juveniles of 2 cultivable shrimp species *P. monodon* and *P. indicus* in the laboratory.

To test the virulence, 18 h old culture of *V. harveyi* isolated from sea water (SW) and haemolymph of *P. indicus* (HS) grown in sea water nutrient broth were resuspended in the sterile saline solution (salinity 20 ppt) and individual shrimps were injected intramuscularly (i.m.) in the second abdominal segment at 10, 10^2, 10^3, 10^4, 10^5 and 10^6 colony forming units (cfu)/shrimp. Ten juveniles each of test shrimps were kept individually in troughs of 1 litre capacity containing 600 ml of sterile sea wa-

ter under constant aeration. The tests were performed in duplicate. The control individuals were injected with sterile saline solution. Shrimp behaviour and mortalities were observed at the end of every 12 h for 96 h.

In the present study, the sea water strain caused 100% mortality at the end of 48 h in *P. monodon* at 10^5 cfu/shrimp; while 100% mortality in *P. indicus* was observed at the end of 84 h. However, the HS strain caused 100% mortality at the end of 48 h in *P. monodon* at 10^3 cfu/shrimp and at the end of 84 h in *P. indicus* (Tables 1 and 2). This indicates that the isolates from the shrimp source are highly pathogenic to shrimps compared to the isolates from sea water. Further, the study also shows that the juveniles of *P. monodon* are more susceptible to *V. harveyi* than the juveniles of *P. indicus*. Hence, *P. indicus* appears to be the sturdier species for culture in India.

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ACKNOWLEDGEMENT. We thank the Director of Instruction, College of Fisheries, Mangalore for the facilities provided.

K. RAVI
N. JAYABALAN
K. ABOKARE
T. M. ANIL

Department of Fisheries Resources and Management,
University of Agricultural Sciences,
College of Fisheries,
Mangalore 575 002, India