Vol. 57: 151–155, 2003 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published December 3

NOTE

Effect of potassium permanganate stress on immune resistance and susceptibility to *Lactococcus garvieae* in the giant freshwater prawn *Macrobrachium rosenbergii*

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ABSTRACT: This work is part of a continuing series of investigations on the effect of commonly used aquaculture chemicals on the immune resistance and susceptibility of the giant freshwater prawn *Macrobrachium rosenbergii* to *Lactococcus garvieae*. The methodology has been described in earlier publications of the series. Potassium permanganate at 1.0 mg l^{-1} in tryptic soy broth (TSB) had no effect on the growth rate of *L. garvieae*. The mortality of *M. rosenbergii* challenged with 4×10^6 colony-forming units (cfu) $prawn^{-1}$ of TSB-grown *L. garvieae* was significantly greater than that of challenged controls. Addition of potassium permanganate at 1.0 mg l^{-1} in TSB significantly increased the virulence of *L. garvieae* to *M. rosenbergii*. Exposure of *M. rosenbergii* to potassium permanganate prior to challenge with TSB-grown *L. garvieae* at 4×10^6 and 3×10^6 cfu $prawn^{-1}$ revealed that 96 h mortality was significantly lower for prawns held in water containing 0.3 mg l^{-1} of the chemical than for prawns in water containing 1.0 mg l^{-1} or no chemical. Potassium permanganate caused no significant changes in total hemocyte counts and differential hemocyte counts, compared to the control treatments. However, a concentration of 1.0 mg l^{-1} or more for 96 h resulted in decreased phenoloxidase activity, phagocytic activity and clearance efficiency. Respiratory burst increased with exposure to 0.3 mg l^{-1}. In conclusion, treatment with potassium permanganate at 0.3 mg l^{-1} was effective in reducing *M. rosenbergii* mortality from *L. garvieae* infection, but higher concentrations had a negative effect, probably due to reduced prawn defenses.

KEY WORDS: *Macrobrachium rosenbergii* · *Lactococcus garvieae* · Potassium permanganate · Hemocyte count · Phenoloxidase activity · Respiratory burst · Phagocytosis

INTRODUCTION

This work is part of a continuing series of investigations examining the effects of commonly used aquaculture chemicals on the immune resistance and susceptibility of the giant freshwater prawn *Macrobrachium rosenbergii* to *Lactococcus garvieae* (Cheng & Wang 2001, Cheng et al. 2002a,b, 2003).

Potassium permanganate is an oxidant of organic and inorganic substances in pond water that is very effective in disinfection and detoxification (Lay 1971, Baticados & Paclibare 1992). Its application dosage in aquaculture varies from 4 to 8 mg l^{-1} (Boyd 1990), but prawn farmers may apply excessive amounts, causing concern about the chemical's potential effect on the disease resistance and immune function of cultured prawns. The 24 h LC_{50} (median lethal concentration) of potassium permanganate on *Macrobrachium rosenbergii* postlarvae is reported to be 6.0 mg l^{-1} (Liao & Guo 1990), but little is known about its effect on prawn resistance or on bacterial virulence.

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The objectives of this study were to examine the effects of potassium permanganate on the growth and virulence of *Lactococcus garvieae*, the resistance of *Macrobrachium rosenbergii* to *L. garvieae*, and the effect of potassium permanganate on immune parameters of *M. rosenbergii*.

MATERIALS AND METHODS

The methods and protocols for this work were the same as those described previously (Cheng & Wang 2001, Cheng & Chen 2002, Cheng et al. 2002a,b, 2003). Briefly, *Macrobrachium rosenbergii* prawns (10 to 12 g) in the intermolt stage were obtained from a commercial farm in Pingtung, Taiwan, and acclimated for 2 wk before experimentation. For bacterial challenge experiments, test and control groups comprised 10 prawns, each in triplicate. After injection, each group was maintained in a separate 60 l glass aquarium containing 40 l aerated water.

For immune parameter assays, prawns weighed 20 to 28 g and were in the intermolt stage. Activity assays were carried out in triplicate or quadruplicate with test groups consisting of 2 prawns each in separate 60 l glass aquaria containing 40 l aerated water. In all tests, prawns were fed twice daily with a formulated prawn diet (Shinta Feed Company, Pingtung, Taiwan). During experiments, the water temperature was maintained at 28 ± 1°C, pH 7.3 to 7.8 and the total water hardness was 100 mg l^{-1}.

Test solutions of *Lactococcus garvieae* were cultured as previously described (Cheng et al. 2002a,b, 2003). The stock solution of potassium permanganate (1000 mg l^{-1}) was made in distilled water. Potassium permanganate was used for tests on growth and virulence at 0 (control) 0.3, 0.6 and 1.0 mg l^{-1} in 250 ml flasks containing 50 ml tryptic soy broth (TSB, Difco) at 30°C for 12, 24, 48 and 120 h. Growth was measured by optical density (OD) at a wavelength of 601 nm using a Model U-2000 spectrophotometer (Hitachi, Tokyo, Japan).

Lactococcus garvieae was pelleted for challenge tests by centrifugation at 7155 × *g* for 15 min at 4°C and re-suspended in saline (0.85% NaCl) at 2 × 10^8 cfu ml^{-1}. This stock bacterial suspension was diluted as appropriate with saline, and injected (20 µl) into the ventral sinus of prawns. Prawns injected with an equal volume of sterile saline solution served as unchallenged controls.

Bath exposure to potassium permanganate was performed as previously described for other test chemicals (Cheng et al. 2003) at 0 (control) and 0.3, 0.6 and 1.0 mg l^{-1}. Test solutions were renewed daily, and experiments ran for 144 h as described previously (Cheng et al. 2003). Hemolymph (100 µl) for measurement of immune parameters was sampled individually at the beginning of the test (0 h) and again at 48 and 96 h. Total hemocyte counts (THC), differential hemocyte counts (DHC), phenoloxidise activity, respiratory burst, phagocytic activity and clearance efficiency were measured.

A multiple comparison (Tukey) test was conducted to examine significant differences among treatments using SAS computer software (SAS Institute). Percent data (virulence and susceptibility studies) were normalized using arcsine transformation before analysis. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Lactococcus garvieae grew equally well in the TSB medium with (0.3 to 1.0 mg l^{-1}) or without potassium permanganate. In challenge tests using these cultured bacteria, all control (unchallenged) prawns survived, but death began to occur in bacterial injected prawns at 24 h. Cumulative mortality after 96 h was higher using bacteria incubated in TSB containing potassium permanganate at 1.0 mg l^{-1} (Table 1).

Media or environmental parameters can affect the growth of pathogens and production of bacterial toxins (Weinberg 1985, Arp 1988). Weinberg (1985) reported that media containing iron and manganese affected

Table 1. Susceptibility of *Macrobrachium rosenbergii* to *Lactococcus garvieae* incubated in TSB (tryptic soy broth) of different potassium permanganate concentrations at 28 ± 1°C, pH 7.3 to 7.8. Data with different letters in same column are significantly different ($p < 0.05$). Values are means ± SD. n = no. of prawns

Bacterial dose (cfu $prawn^{-1}$)	Ambient $KMnO_4$ (mg l^{-1})	n	Cumulative mortality (%)					
			24	48	72	96	120	144
Control	Saline	30	0	0	0	0	0	0
4×10^6	0	30	7 ± 6^b	17 ± 0^b	23 ± 6^b	23 ± 6^b	23 ± 6^b	23 ± 6^b
4×10^6	0.3	30	10 ± 0^b	20 ± 0^b	23 ± 12^b	27 ± 12^{ab}	27 ± 12^{ab}	27 ± 12^{ab}
4×10^6	0.6	30	7 ± 6^b	27 ± 6^{ab}	30 ± 10^{ab}	30 ± 10^{ab}	30 ± 10^{ab}	30 ± 10^{ab}
4×10^6	1.0	30	23 ± 6^a	37 ± 6^a	43 ± 6^a	43 ± 6^a	47 ± 12^a	47 ± 12^a

the growth of pathogens, and modulated the yields of bacterial enzymes and toxins. Siegel (1981) reported that in complex media, the optimal iron concentration for maximal production of the neurotoxins of *Clostridium botulinum* was 5.4 µM. Previously, we have shown that ammonia, nitrite and copper sulfate also affected the growth and virulence of *Lactococcus garvieae* (Cheng & Wang 2001, Cheng & Chen 2002, Cheng et al. 2002a). These earlier results showed that copper sulfate at 0.4 mg l^{-1} and potassium permanganate at 1.0 mg l^{-1} increased the virulence of *L. garvieae* to *M. rosenbergii*, while ammonia-N at 0.26 mg l^{-1} and nitrite-N at 1.5 mg l^{-1} decreased the virulence.

With respect to *Macrobrachium rosenbergii* bathed in potassium permanganate, all unchallenged control prawns survived. However, challenges with *Lactococcus garvieae* at 4×10^6 cfu $prawn^{-1}$ after prawn exposure to 0.3 mg l^{-1} potassium permanganate resulted in a significantly later (24 h) onset of mortality ($p < 0.05$) than for prawns exposed to 0, 0.6 or 1.0 mg l^{-1} (16 h). After 72 to 144 h, cumulative mortality (Table 2) of prawns exposed to 0.3 mg l^{-1} was significantly ($p < 0.05$) lower (27%) than in the unexposed control (40%) or other treatment (50 to 63%) groups. Reducing the challenge dose to 3×10^6 cfu $prawn^{-1}$ (data not shown) removed significant differences in cumulative mortality at 144 h, except for the comparison between the 0.3 mg l^{-1} group (27%) and the 1.0 mg l^{-1} group (43%). Survival for the other 2 groups was 33%.

Previously, we have shown that the susceptibility of *Macrobrachium rosenbergii* to *Lactococcus garvieae* increases at high pH, high temperature (Cheng & Chen 1998), and in the presence of ammonia, nitrite and copper sulfate (Cheng & Wang 2001, Cheng & Chen 2002, Cheng et al. 2002a).

Bath exposure to potassium permanganate produced no significant differences in THC (total hemocyte counts) and DHC (differential hemocyte counts) compared to untreated control prawns. The mean ± SE THC varied from $112 \pm 19 \times 10^5$ to $208 \pm 60 \times 10^5$ cells ml^{-1}. The mean ± SE DHC varied from $100 \pm 19 \times 10^5$ to $194 \pm 27 \times 10^5$ for hyaline cells, $5 \pm 3 \times 10^5$ to $8 \pm 4 \times 10^5$ for semi-granular cells, and $4 \pm 2 \times 10^5$ to $7 \pm 4 \times 10^5$ for granular cells. However, chemical exposure significantly ($p < 0.05$) decreased phenoloxidase activity (Fig. 1) by 23% at 48 h to 43% at 96 h. In contrast, respiratory burst increased with increasing exposure time at all potassium permanganate concentrations (Fig. 2) to a maximum of 29% at 96 h in the 1.0 mg l^{-1} exposure group.

We previously examined the potential effect of extrinsic factors on circulating hemocyte numbers and on the immune parameters of decapod crustaceans (Cheng & Chen 2000, 2002, Cheng & Wang 2001, Cheng et al. 2002a,b). Similar to our earlier results with ammonia, nitrite and copper sulfate (Cheng & Wang 2001, Cheng & Chen 2002, Cheng et al. 2002a), we found no differences with potassium permanganate, in the present study. Also similar to our previous work with copper sulfate (Cheng & Wang 2001) and ammonia (Cheng & Chen 2002), in the present study phenoloxidase activity decreased after 96 h exposure to potassium permanganate (0.3 to 1.0 mg l^{-1}). Thus, in all

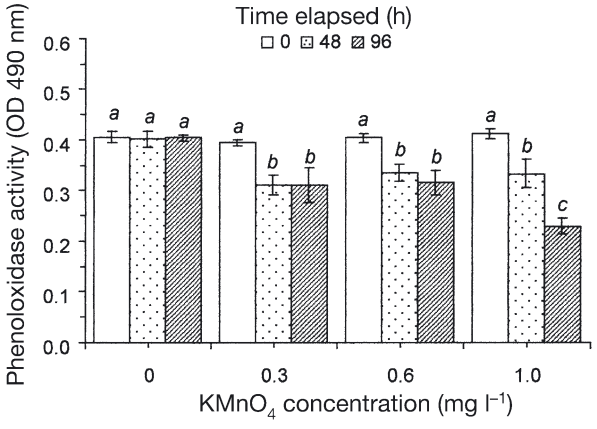

Fig. 1. *Macrobrachium rosenbergii.* Mean (±SE) phenoloxidase activity after 0, 48 and 96 h exposure to different concentrations of potassium permanganate. Each bar represents mean of 6 measurements. Bars with different letters for same potassium permanganate concentration are significantly different ($p < 0.05$)

Table 2. Susceptibility of *Macrobrachium rosenbergii* to *Lactococcus garvieae* at different concentrations of potassium permanganate. Data with different letters in same column are significantly different ($p < 0.05$) among treatments. Values are means ± SD. n = no. of prawns

Bacterial dose (cfu $prawn^{-1}$)	Ambient $KMnO_4$ (mg l^{-1})	n	Cumulative mortality (%)						
			16	24	48	72	96	120	144
Control	1.0	30	0	0	0	0	0	0	0
4×10^6	0	30	10 ± 10^{ab}	23 ± 6^{ab}	33 ± 12^{ab}	40 ± 0^{b}	40 ± 0^{b}	40 ± 0^{b}	40 ± 0^{b}
4×10^6	0.3	30	0 ± 0^{b}	13 ± 6^{b}	23 ± 6^{b}	27 ± 6^{c}	27 ± 6^{c}	27 ± 6^{c}	27 ± 6^{c}
4×10^6	0.6	30	3 ± 6^{ab}	27 ± 6^{ab}	50 ± 10^{a}	53 ± 6^{a}	57 ± 6^{a}	57 ± 6^{a}	57 ± 6^{a}
4×10^6	1.0	30	13 ± 6^{a}	33 ± 6^{a}	50 ± 0^{a}	50 ± 0^{a}	57 ± 6^{a}	60 ± 10^{a}	63 ± 12^{a}

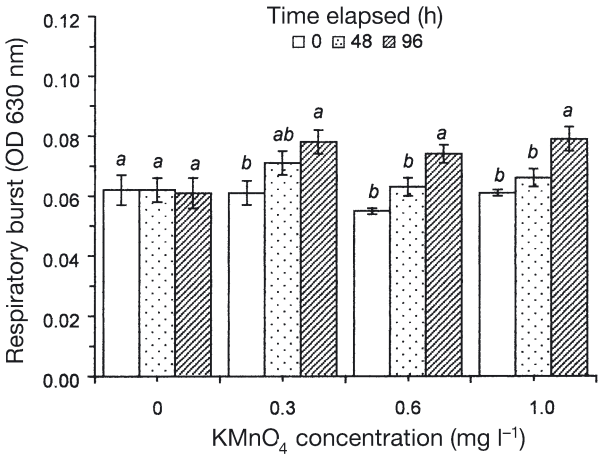

Fig. 2. *Macrobrachium rosenbergii.* Mean (±SE) respiratory burst after 0, 48 and 96 h exposure to different concentrations of potassium permanganate. Further details as in Fig. 1

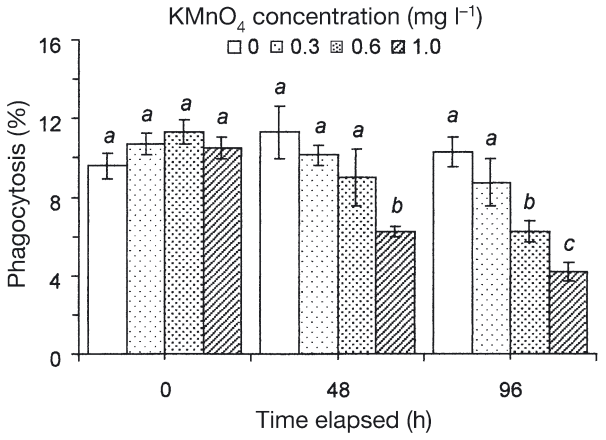

Fig. 3. *Macrobrachium rosenbergii.* Mean (±SE) phagocytic activity after 0, 48 and 96 h exposure to different concentrations of potassium permanganate. Each bar represents mean of 8 measurements. Bars with different letters for same exposure time are significantly different ($p < 0.05$)

our similar studies, the recorded decrease in phenoloxidase activity was not a consequence of reduced THC or altered DHC.

The increased release of superoxide anion upon exposure to potassium permanganate in the present study was also similar to our earlier results with ammonia, nitrite and copper sulfate (Cheng & Wang 2001, Cheng & Chen 2002, Cheng et al. 2002a), whereby moderate concentrations increased immunity and high concentrations caused cytotoxicity (Muñoz et al. 2000). Further research on activities of superoxide dismutase, catalase and peroxidase (Holmblad & Söderhäll 1999) are necessary to explain the production of ROIs (reactive oxygen intermediates) under potassium permanganate stress.

There was a small (4 to 6%) but significant decrease in phagocytic activity during exposure to 0.6–1.0 mg l^{-1} potassium permanganate at 96 h compared to unexposed controls (Fig. 3). In contrast, larger decreases in *Lactococcus garvieae* clearance efficiency were observed (Fig. 4) by 48 h at 0.6 mg l^{-1} (47%) and 1.0 mg l^{-1} (147%). The largest decrease of 155% occurred at 96 h in 1.0 mg l^{-1}. In an earlier study, we also showed that both phagocytic activity and clearance efficiency to *L. garvieae* decreases after exposure to nitrite-N (Cheng et al. 2002a).

In conclusion, our results suggest that 0.3 mg l^{-1} potassium permanganate in aquaculture ponds effectively reduces mortality of *Macrobrachium rosenbergii* due to *Lactococcus garvieae* infection, whereas higher levels are detrimental. The reason for the reduced mortality is unknown, but it was not due to any inhibitory effect on *L. garvieae* growth or to any improvement in the prawn immune parameters we measured.

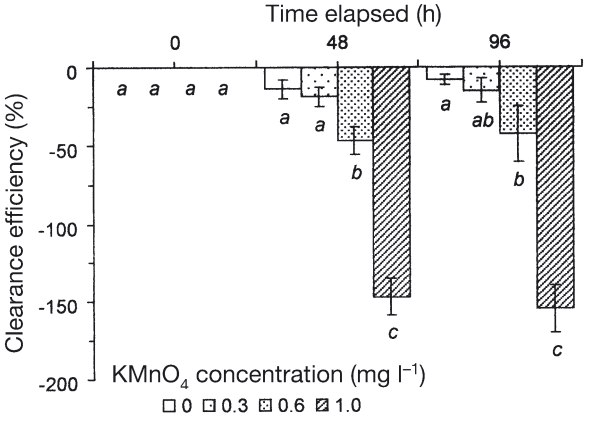

Fig. 4. *Macrobrachium rosenbergii.* Mean (±SE) clearance efficiency during exposure to different concentrations of potassium permanganate. Further details as in Fig. 3

Acknowledgements. This work was supported by a grant from the Council of Agriculture of the Republic of China (COA 89-1.2-04-29). We thank Dr. S. C. Sheu, Associate Professor of Department of Food Science for the use of the ELISA reader, Mr. C. H. Chang and Mr. C. H. Liu for their assistance during the experiment, and Mr. Y. H. Chen, University of California, Berkeley, for his critical review of the manuscript.

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Editorial responsibility: Timothy Flegel, Bangkok, Thailand

Submitted: September 23, 2002; Accepted: July 9, 2003
Proofs received from author(s): September 22, 2003