Original Article

Identification and Pathology of *Lactococcus garvieae* Isolated from Cultured and Wild Giant Freshwater Prawns (*Macrobrachium rosenbergii* de Man) in Thailand

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Abstract

Lactococcosis caused by *Lactococcus garvieae* is an increasing problem in aquaculture. The current study is, to our knowledge, the first record of *L. garvieae* isolated from giant freshwater prawn (*Macrobrachium rosenbergii* de Man) in Thailand. These bacteria were isolated from giant freshwater prawns that were either cultured or in their natural environments in Phatthalung and Songkhla, provinces of southern Thailand. Based on conventional and rapid identification systems, as well as genetic and phylogenetic characterizations, the bacteria were identified as *L. garvieae*. An infectivity trial indicated that all *L. garvieae* isolates were pathogenic bacteria. Moreover, giant freshwater prawns experimentally infected with *L. garvieae* KSAAHRC-LA1, by intramuscular injection, exhibited 23.33–86.67% mortality within 10 d and a 10-day LD_{50} of 1.38 x 10^7 CFU/ml. Histopathological findings revealed severe damaged hepatopancreatic tubules caused by bacterial infection and hemocytic infiltrations observed in the infected muscle. Haemato-immunological parameters of the giant freshwater prawn infected with *L. garvieae* indicated depression in the prawn's immune response.

Keywords: characterization, *Lactococcus garvieae*, *Macrobrachium rosenbergii*, pathogenicity

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Introduction

Lactococcal infections in aquatic animals have become an increasing issue in aquaculture. The dominant organism responsible for lactococcosis in aquatic animals is *Lactococcus garvieae*, which is an emerging zoonotic agent isolated from humans (Fefer et al., 1998; James et al., 2000; Wang et al., 2007), dolphins (Evans et al., 2006), various economically important fishes (Eldar and Ghittino, 1999; Ooyama et al., 1999; Vela et al., 2000; Chen et al., 2002; Baeck et al., 2006), and prawns (Cheng and Chen, 1998; Chen et al., 2001).

Giant freshwater prawn (*Macrobrachium rosenbergii* de Man) is a native prawn of Thailand and other Southeast Asian countries (New, 1990). This prawn is commercially cultured as well as captured in the wild, in Thailand, but the natural catch has drastically declined (Tonguthai, 1997), due to excessive fishing and deterioration of its habitat and spawning grounds. The rising demand for prawns has caused a significant increase in the number of prawn farms. However, prevalent infectious diseases damage the prawn cultures. The major causative agents for diseases in giant freshwater prawns include protozoa, fungi, viruses and bacteria (Tonguthai, 1997; Cheng and Chen, 1998; Yoganandhan et al., 2006; Chen et al., 2001).

The bacterial disease caused by *L. garvieae* has caused great losses of giant freshwater prawns in Taiwan (Cheng and Chen, 1998; Chen et al., 2001). In the current study, we report the phenotypic and biochemical characteristics, and genetic and phylogenetic characterizations of *L. garvieae* isolated from giant freshwater prawns, sampled from both cultured and natural environments. In addition, the histopathological changes and immune responses of giant freshwater prawn infected by *L. garvieae* were investigated, along with the pathogenicity of infection.

Materials and Methods

Bacteriology: Stage, origin, and culture system of giant freshwater prawn samples collected for bacterial isolation are summarized in Table 1. Bacterial isolates were obtained from giant freshwater prawns cultured and captured in 6 areas in Phatthalung and Songkhla provinces of southern Thailand.

Samples from hepatopancreas of adult or broodstock giant freshwater prawns were taken, and streaked onto tryptic soy agar (TSA: Difco) using aseptic techniques. Giant freshwater prawn larvae were placed on a sterile filter net rinsed with sterile seawater and sprayed with 70% ethanol to eliminate fouling pathogens. The ethanol was washed off with sterile seawater, and the larvae were immediately macerated and homogenized in a sterile mortar with a pestle. The samples were then taken and streaked onto TSA. After incubation at 30°C for 24-48 h, single presumptive colonies selected from almost pure or dominant colonies on TSA were picked for Gram-staining and test for catalase activity. Twenty-eight purified bacterial isolates of catalase negative and Gram positive cocci were inoculated into tryptic soy broth (TSB: Difco), supplemented with 15% glycerol, and stored at -70 °C.

Biochemical characterization of bacterial isolates: Eighteen purified bacterial isolates, including selected isolates from each sampling site were characterized biochemically. Biochemical reactions of isolates were assessed on API20STREP systems (bioMérieux®, Marcy l' Etoile, France) according to the manufacturer's instructions. Conventional characterizations of the bacteria were done, namely Gram staining reaction, oxidase test, catalase test, and haemolytic properties on blood agar supplemented with 5% defibrinated sheep blood. The conventional and API20STREP tests were quality controlled and validated using a known isolate of *L. garvieae* FK040708. The classification method described by Hawke (2000) was used to determine bacterial genus and species.

Multiplex polymerase chain reaction (m-PCR): To confirm the identity of *L. garvieae* using m-PCR, total nucleic acid was extracted from 18 pure *L. garvieae* cultures using the phenol-chloroform method described by Ausubel et al. (1995). Three sets of oligonucleotide primers, capable of detecting specific sequences of the 16S rRNA gene of *Streptococcus agalactiae* (Martinez et al., 2001), the lactate oxidase-encoding gene (*lctO*) of *Streptococcus iniae* (Mata et al., 2004) and the 16S rDNA gene of *L. garvieae* (Zlotkin et al., 1998) were used. The m-PCR conditions were empirically determined using the method of Itsaro et al. (2012). Amplification of target DNA was conducted on a PTC-100™ thermal cycler (MJ Research Inc., USA). A negative control without template DNA was included in the m-PCR experiments. The m-PCR products were subjected to electrophoresis (30 min, 110V; Mupid®-exu, Japan) in 1.5% agarose gel with TBE buffer (90 mM Tris, 90 mM borate, and 2 mM ethylenediamine tetraacetic acid, pH 8.0) and visualized by ethidium bromide staining. The DNA marker was a 100 bp ladder (Vivantis).

Table 1 Stage, origin, and culture system of prawn samples collected for *L. garvieae* isolation

Date	Stage	Origin	Culture system
Jun 09	Post larva	Sathing Phra, Songkhla	Concrete pond/Hatchery
Dec 09	Adult	Paphayom, Phatthalung	Earthen pond/Prawn farm
Oct 10	Broodstocks	Muang, Phatthalung	Earthen pond/Hatchery
Jan 11	Adult	Songkhla lake, Khuanniang, Songkhla	Wild catch
May 11	Adult	Songkhla lake, Pakpayoon, Phatthalung	Wild catch
May 11	Adult	Songkhla lake, Krasae Sin, Songkhla	Wild catch

Phylogenetic analysis: The amplified product obtained from the m-PCR assay, derived from *L. garvieae* KSAAHRC-LA1 sample, was purified with a Gel Extraction Kit (Qiagen) according to the manufacturer's protocol, and sequenced on an Applied Biosystems Genetic Analyzer using PCR primers pLG-1 and pLG-2.

The Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov) matched the sequences generated with homologous sequences in the Entrez database. Thirty homologous *L.garvieae, Lactococcus fujiensis, Lactococcus lactis, Lactococcus piscium, Lactococcus plantarum, Lactococcus chungangensis,* and *Lactococcus raffinolactis* sequences were chosen for phylogenetic analysis, using the clustalW application of the Molecular Evolutionary Genetic Analysis (MEGA) package (version 4). The analysis included *S. iniae* as an out-group species. An un-rooted evolutionary tree was inferred using the neighbor-joining (N-J) tree algorithm. Resultant tree topologies were generated by bootstrap analysis of the N-J method based on 1000 replicates.

Experimental animals: Five hundred healthy giant freshwater prawns, with an average weight of 15.00 $\pm$ 4.98g, were maintained in a 3 metric ton fiber glass tank at Kidchakan Supamattaya Aquatic Animal Health Research Center (KSAAHRC). Aeration was supplied through air stones, and temperature was maintained at 26-28^{o}C. The prawns were given commercial feed daily at 3-5% of body weight during acclimation period. The prawns were examined to ensure that they were free from disease prior to use in experimental trial.

Pathogenicity study: Pathogenicity of 18 *L. garvieae* isolates obtained from giant freshwater prawns was determined using a method modified from Wanman et al. (2005). The experiment was conducted in nineteen 50x120x50 cm glass aquariums containing 250 L dechlorinated water with aeration. Five prawns were infected per bacterial isolate, by intramuscular injection with 0.1 ml of bacterial suspension in PBS (pH7.4) containing 10^8 CFU/ml (equal to 0.5 MacFarland Standard and optical density of 1.0 at 600 nm wavelength). A control group was similarly inoculated with sterile PBS. Mortality of prawns was monitored daily for 10 d, and from its final level virulence of isolates were assessed and labeled as follows: mortality of at least 4 prawns (strongly virulent); mortality of 2-3 prawns (virulent); mortality of 1 prawn (weakly virulent) and no mortality (avirulent).

Lethal dose (LD_{50}): The degree of virulence of purified bacteria was determined in terms of the lethal dose (LD_{50}) value in giant freshwater prawns. *L. garvieae* isolate KSAAHRC-LA1, which was originally isolated from giant freshwater prawn post larvae cultured in a hatchery in Songkhla Province, was chosen for this pathogenicity study because it had produced high mortality in giant freshwater prawns. Sixteen h culture of *L. garvieae* KSAAHRC-LA1 in TSB was pelleted by centrifugation at 10,000 rpm for 10 min at 4°C. The cells were washed twice with PBS (pH7.4), adjusted to an optical density of 1.0 at 600 nm wavelength (equal to 10^8 CFU/ml) and diluted to achieve final concentration of approximately 1 x 10^6, 5 x 10^6, 1 x 10^7 and 5x10^7 CFU/ml. Viable bacterial count were determined using the drop plating serial dilution technique on TSA. The subject prawns were given an intramuscular injection with 0.1 ml of each suspension of *L. garvieae* KSAAHRC-LA1. The experiment was conducted in triplicate using 10 prawns per glass aquarium (i.e. 30 prawns/dose level). A control group was similarly inoculated with sterile PBS. Mortalities were recorded twice daily for a 10-day period. Signs of moribund and dead prawns were observed, and a probit analysis program (Luangthuvapranit, 1988) was used to estimate the LD_{50} value. Hepatopancreases were collected from all moribund prawns, and bacterial cells were re-isolated on TSA to confirm *L. garvieae* as the cause of death.

Sampling procedure for histopathology: Tissue samples were taken from diseased prawns, from both culture environment and those experimentally infected. The tissues collected were hepatopancreas, gill, and muscle, and these were fixed in Davidson's fixative. Examination for histopathological changes in infected prawns followed a standard histological technique (Humason, 1979). After fixation each sample was cut into small pieces for better penetration of the reagents, and embedded in paraffin. Histological sections were made and stained using haematoxylin and eosin (H&E). The stained sections were examined under a light microscope.

Haemato-immunological parameters: One hundred and twenty giant freshwater prawns were used in these experiments. The subject prawns were given an intramuscular injection with 0.1 ml of 8.9x10^6 CFU/ml of *L. garvieae* KSAAHRC-LA1. The experiment was conducted in five replicates using 12 prawns per glass aquarium. A control group was similarly inoculated with sterile PBS. At 0, 1, 3, 6 and 9 d post injection, two prawns were randomly sampled from each replication, and haemolymph was withdrawn by using a 1-ml syringe with 25G needle from the base of a walking leg. Total haemocyte counts were determined following Supamattaya et al. (2000[a]). Phenoloxidase activity was determined using a method modified from Smith and Söderhäll (1983). Glucose level was measured using a method of Hyvarinen and Nikkila (1962). Total haemolymph protein was determined colorimetrically by the method of Lowry et al. (1951), see Supamattaya et al. (2000[a]).

Results

Bacteriology: Clinical observations of the present study indicated that the diseased giant freshwater prawns exhibited an atrophy and pale hepatopancreas. *L. garvieae* were isolated in almost pure cultures from diseased giant freshwater prawns that were post larvae and adults sampled from Sathing Phra, Songkhla and Paphayom, Phatthalung provinces, while small numbers of mixed cultures were isolated from apparently normal broodstocks and adults sampled from Muang and Pakpayoon, Phatthalung province and adults sampled from Khuanniang and Krasae Sin,

Songkhla province. These mixed cultures contained other bacteria including *Enterobacter* spp., *Shewanella putrefaciens, Pasteurella pneumotropica,* and *Klebsiella pneumonia*.

Biochemical characterization of bacterial isolates: Bacterial isolates from giant freshwater prawns were confirmed to be *L. garvieae* using conventional phenotypic characteristics and the API20STREP system (Table 2). The phenotypic characteristics of the isolates were as follows: Gram positive cocci, grouped in pairs or in short chains, catalase negative, oxidase negative, and capable of growing on blood agar medium (5% sheep blood) to produce a α-haemolytic reaction. Eighteen isolates obtained from the giant freshwater prawns displayed similar biochemical characteristics, indicating that they were all *L. garvieae*.

Multiplex polymerase chain reaction and phylogenetic analysis: A specific DNA fragment in the length of 1,100 bp was amplified from template DNA samples. This band was detected in the reference strain known to represent *L. garvieae* and in all of the isolates from the present study assumed to represent *L. garvieae* based on biochemical characteristics.

Phylogenetic analysis based on the 16s rRNA sequences of *L. garvieae* KSAAHRC-LA1 (GenBank Accession No. KF939114) corroborated that this was *L. garvieae*. The sequence was 100% similar to eleven prior isolates of *L. garvieae* stored in GenBank with accession numbers AB598960, JN162117, HM536980, FJ915634, JF811915, AB267905, GU299084, JQ446487, AB244437, EU081016, and HQ721279. The phylogenetic tree (Fig 1) constructed using the neighbor-joining method, clustered the *L. garvieae* KSAAHRC-LA1 strain with the eleven *L. garvieae* GenBank sequences.

Experimental infectivity trial: There was no mortality in the giant freshwater prawn intramuscularly injected with sterile PBS. Most *L. garvieae* isolates were found strongly virulent or virulent for giant freshwater prawn. A single isolate was labeled as weakly virulent (Table 3), namely that obtained from a diseased giant freshwater prawn cultured in Paphayom, Phatthalung province.

The giant freshwater prawns inoculated by intramuscular injection of *L. garvieae* KSAAHRC-LA1 exhibited 23.33-86.67 % mortalities within 10 d, in a dose dependent manner. During early onset of the disease, the infected prawns exhibited more aggressive behavior than those of the control group. After that, the infected prawns became lethargic, swam at the bottom of the aquarium, and finally died. Inoculation and reisolation of *L. garvieae* from the dead prawns confirmed Koch's postulates, and established the organism as a pathogen of giant freshwater prawn. The experimental challenge of giant freshwater prawns gave a 10-day LD_{50} of 1.38 x 10^7 CFU/ml by intramuscular injection. No mortality occurred in the control groups.

Histopathology: Histopathological changes of the giant freshwater prawns infected by *L. garvieae* were observed in the hepatopancreas and muscle. On comparison to normal hepatopancreas and muscle (Figs 2-3), the infected prawns exhibited severe damaged hepatopancreatic tubules caused by bacterial infection (Fig 4). There were hemocytic infiltrations observed in the infected muscle (Fig 5). No histopathological changes were observed in the gill tissues.

Haemato-immunological parameters: Studies on haemato-immunological parameters of the giant freshwater prawns infected with *L. garvieae* KSAAHRC-LA1 showed a reduction in phenoloxidase activity and glucose level at 3, 6, 9 and 1, 3, 6, 9 d post infection, respectively (Figs 7-8). No significant differences were found in the total haemocyte count and protein of haemolymph between the *L. garvieae*-infected and non-infected prawns (Figs 6 and 9).

Table 2 Comparison of the phenotypic characteristics of isolates from giant freshwater prawn (*M. rosenbergii*) assumed to be *L. garvieae* with the reference strain identified as such

Test	Present isolates (n=18)	*L. garvieae* FK040708 (n=1)	Test	Present isolates (n=18)	*L. garvieae* FK040708 (n=1)
Gram staining reaction	+	+	β-Galactosidase	-	-
Cell morphology	Cocci	Cocci	Alkaline phosphatase	-	-
Catalase	-	-	**Acid production from:**		
Oxidase	-	-	- L-arabinose	-	-
Haemolysis	α	α	- D-mannitol	-	-
VP test	+	+	- D-sorbitol	- (94.4)	+
Hippurate	-	-	- D-lactose	- (94.4)	+
Esculin	+	+	- D-trehalose	+	+
Pyrrolidonyl arylamidase	+	+	- Inulin	-	-
Leucine aminopeptidase	+	+	- D-raffinose	-	-
α-Galactosidase	-	-	- Starch	-	+
β-Glucuronidase	-	-	- Glycogen	-	-

Identification: n= reflects number of isolates, + = positive; - = negative; α = alpha-haemolysis; ( ) = denoted percent of isolates that gave the negative result

Table 3 Virulence of *L. garvieae* isolates from diseased and apparently healthy giant freshwater prawns

Virulence level	Number (%) of isolates
Strongly virulent	13(72)
Virulent	4(22)
Weakly virulent	1(6)
Avirulent	0(0)

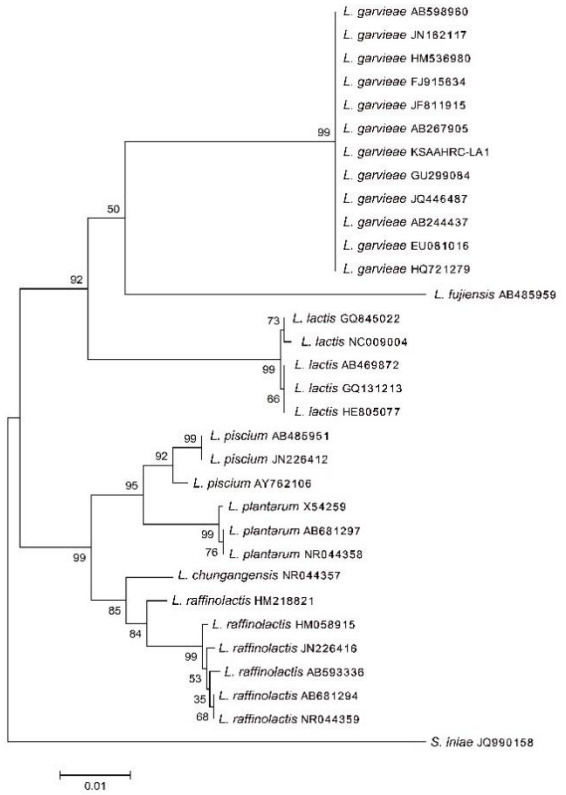

Figure 1 Phylogenetic tree based on 16s rDNA sequences of *L. garvieae* from the present study (*L. garvieae* KSAAHRC-LA1) and homologous sequences in the GenBank database. GenBank accession numbers are shown by names of the strains and the scale bars indicate distances.

Discussion

The taxonomic status of the bacterial isolates was determined using morphological and biochemical characteristics, as well as genetic and phylogenetic analysis. The results suggest that the isolates hypothesized as *L. garvieae* are biochemically and physiologically similar to the *L. garvieae* reference isolate, and the *L. garvieae* reported by Chen et al. (2001). The genus *Lactococcus* would previously have belonged to the family Streptococcaceae. This genus was defined in 1985, after the division of the *Streptococcus* genus, and included a group of agents known as lactic streptococci isolated from dairy cattle and milk products (Schleifer et al., 1985). The bacterium *L. garvieae* (Synonym: *Enterococcus seriolicida*), previously described as *Streptococcus garvieae*, was originally isolated in the United Kingdom from a mastitic udder (Collins et al., 1983). In 1991, it was proposed as a new species, E. *seriolicida*, in order

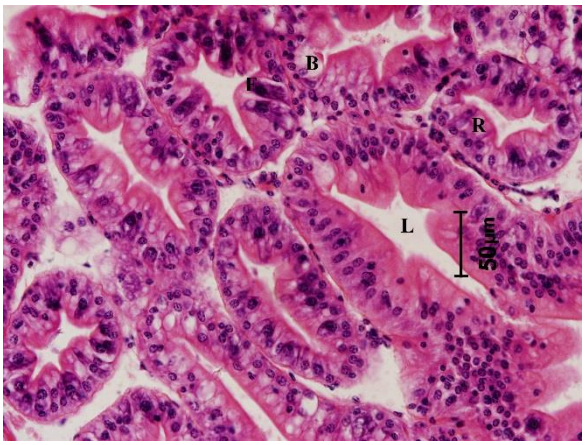

Figure 2 Histological section of normal hepatopancreatic tissue from healthy giant freshwater prawn. B = B-cell, R = R-cell F = F-cell, L= hepatopancreatic tubule lumen (H&E, Bar= 50 µm).

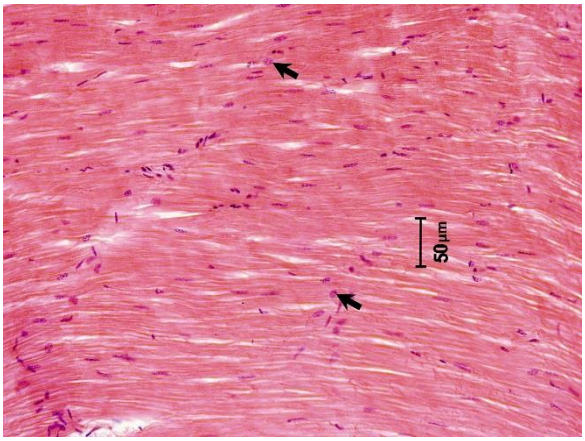

Figure 3 Histological section of normal muscle tissue from healthy giant freshwater prawn. Arrow = hemocyte (H&E, Bar= 50 µm).

to bring together a number of Gram-positive bacteria isolated from streptococcosis outbreaks in Japanese yellowtail over the preceding 20 years (Kusuda et al., 1976; Kusuda et al., 1991). Although this bacterium was classified as *E. seriolicida* (Kusuda et al., 1991), recent reports suggest that *E. seriolicida* should be reclassified as a junior synonym of *L. garvieae* based on its biochemical API20STREP and API50CH characteristics and DNA-DNA hybridization (Eldar et al., 1996).

L. garvieae in the present study were isolated from various sources including diseased and apparently normal prawns. Although these bacteria are not abundant in normal prawns and seem to be an opportunistic pathogen, our study indicated that all *L. garvieae* isolates showed pathogenicity markers irrespective of their origin (diseased or normal prawns), and the challenge experiments fulfilled Koch's postulates and confirmed that all 18 *L. garvieae* isolates were pathogenic to giant freshwater prawn.

Crustacean hemocytes play important roles in the host immune response. Based on the presence of cytoplasmic granules, the hemocyte can be divided into three types, i.e. hyaline cells, semigranular cells, and granular cells (Johansson et al., 2000). Each cell

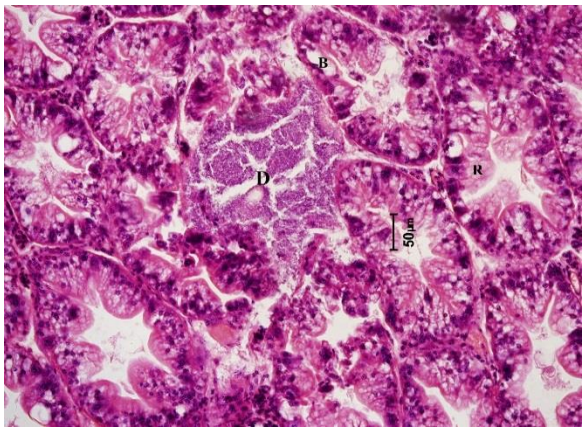

Figure 4 Histological section of hepatopancreatic tissue from an infected giant freshwater prawn showing damaged hepatopancreatic tubule (D) caused by bacterial infection. B = B-cell, R = R-cell (H&E, Bar= 50 µm).

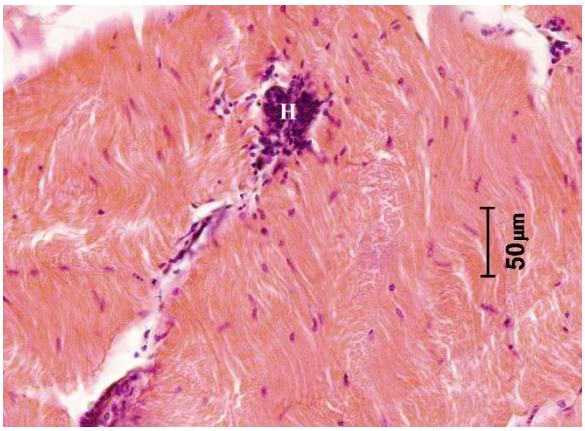

Figure 5 Histological section of muscle tissue from an infected giant freshwater prawn showing hemocytic infiltrations in the damaged muscle; H= hemocyte (H&E, Bar= 50 µm).

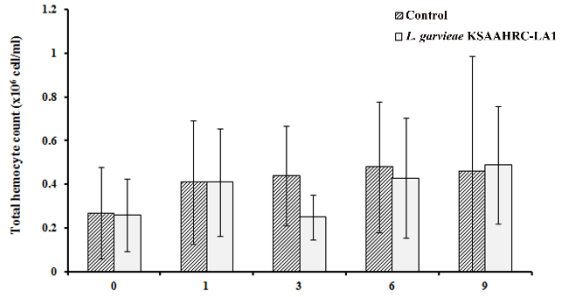

Figure 6 Total hemocyte count of giant freshwater prawn at 0, 1, 3, 6 and 9 d post infection with *L. garvieae* KSAAHRC-LA1

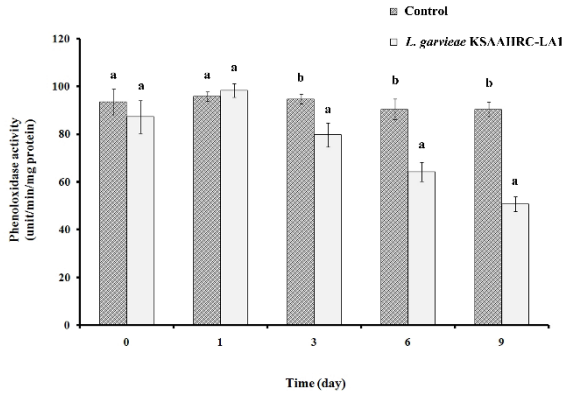

Figure 7 Phenoloxidase activity of giant freshwater prawn at 0, 1, 3, 6 and 9 d post infection with *L. garvieae* KSAAHRC-LA1. Times for control and *L. garvieae* KSAAHRC-LA1 with different letter represent significant differences ($p<0.05$).

type is active in defense mechanisms, for example, the hyaline cells are involved in phagocytosis. The semigranular cells are active in encapsulation, and the granular cells participate in storage and release of the prophenoloxidase system and cytotoxicity (Söderhäll and Cerenius, 1992; Johansson et al., 2000). The prophenoloxidase activating system has been recognized as an efficient innate immune response against microbial pathogens. Glucose value has also been reported as an indicator for evaluating the physiological status of cultured shrimp (Pascual et al., 2003). Reductions in PO activity and glucose level after bacterial and viral infections have been reported in black tiger shrimp (*Peneaus monodon*), white shrimp (*Litopenaeus vannamei*) and kuruma shrimp (*Marsupenaeus japonicus*) (Supamattaya et al., 2000[b]; Yeh et al., 2009; Chen et al., 2011). In the current study the haemato-immunological parameters of giant freshwater prawn infected with *L. garvieae* had decreased phenoloxidase activity and glucose level, indicating that *L. garvieae* depressed the immune response and physiological status of giant freshwater prawns.

L. garvieae is a rare pathogen with low virulence for humans. Members of the genus *Lactococcus* are not abundant in the normal human flora, and seem to behave like opportunistic pathogens in elderly immunodeficient subjects and individuals with prosthetic valves (Fihman et al., 2006). Moreover, the sporadic occurrences of human *L. garvieae* infections appear to correlate with the seasonal aquaculture outbreaks of *L. garvieae* infections (Wang et al., 2007). Further studies of *L. garvieae* isolates from nearby agricultural sources, such as prawn and fish, as well as human isolates, might provide a deeper understanding of the epidemiology of these bacteria in humans and animals.

The present study demonstrated strong association of *L. garvieae* infections to the health and mortality of giant freshwater prawns. However, the current study was limited in its scope to laboratory conditions, aside from initial sample collection. Further investigation into the effects of environmental factors in pond farming of prawns is necessary to elucidate the pathogenicity of these bacteria, and to study the effects of other bacteria such as *Enterobacter* spp. on prawns.

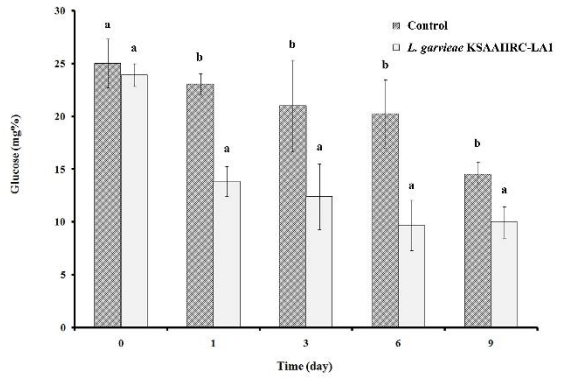

Figure 8 Glucose level of giant freshwater prawn at 0, 1, 3, 6 and 9 d post infection with *L. garvieae* KSAAHRC-LA1. Times for control and *L. garvieae* KSAAHRC-LA1 with different letter represent significant differences ($p<0.05$).

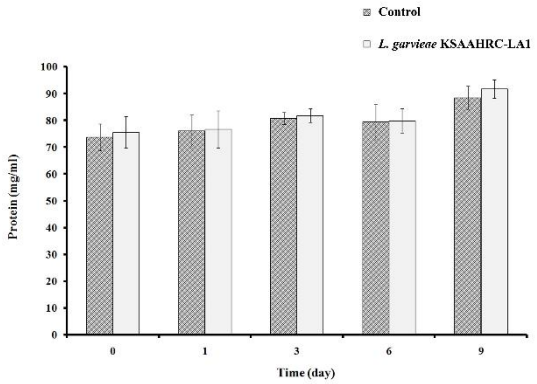

Figure 9 Protein level of giant freshwater prawn at 0, 1, 3, 6 and 9 d post infection with *L. garvieae* KSAAHRC-LA1

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บทคัดย่อ

การจำแนกชนิดและพยาธิวิทยาของแบคทีเรีย *Lactococcus garvieae* ที่แยกได้จากกุ้งก้ามกราม (*Macrobrachium rosenbergii* de Man) ที่เลี้ยงและจับจากธรรมชาติในประเทศไทย

นเรศ ซ่วนยุก* มัชลิน แดงเวชงาม

โรคแลคโตคอคโคซีสที่มีสาเหตุจากแบคทีเรีย *Lactococcus garvieae* สร้างปัญหาอย่างมากต่อการเพาะเลี้ยงสัตว์น้ำ การศึกษาครั้งนี้เป็นรายงานครั้งแรกของการติดเชื้อแบคทีเรีย *L. garvieae* ในกุ้งก้ามกราม (*Macrobrachium rosenbergii* de Man) ในประเทศไทย โดยสามารถแยกแบคทีเรียชนิดนี้ได้จากกุ้งก้ามกรามทั้งที่เลี้ยงและจับจากธรรมชาติในจังหวัดพัทลุงและจังหวัดสงขลา การจำแนกชนิดโดยอาศัยคุณสมบัติทางชีวเคมีร่วมกับการใช้ชุดทดสอบและการทำ phylogenetic analysis พบเป็นแบคทีเรีย *L. garvieae* การทดลองติดเชื้อในกุ้งก้ามกรามพบว่าแบคทีเรีย *L. garvieae* เป็นแบคทีเรียก่อโรค และการทดสอบความรุนแรงของแบคทีเรีย *L. garvieae* KSAAHRC-LA1 โดยการฉีดเข้ากล้ามเนื้อ พบว่าแบคทีเรีย *L. garvieae* ทำให้กุ้งก้ามกรามตาย 23.33–86.67 เปอร์เซ็นต์ ภายในเวลา 10 วัน และมีค่า 10 – day LD_{50} เท่ากับ 1.38×10^7 ซีเอฟยู/มล. การศึกษาการเปลี่ยนแปลงทางพยาธิสภาพของเนื้อเยื่อกุ้งก้ามกรามที่ติดแบคทีเรีย *L. garvieae* พบท่อตับและตับอ่อนถูกทำลายเนื่องจากการติดเชื้อ และพบการแทรกตัวของเม็ดเลือดในกล้ามเนื้อกุ้งที่ติดเชื้อ การศึกษาการเปลี่ยนแปลงองค์ประกอบเลือดของกุ้งก้ามกรามที่ติดเชื้อ *L. garvieae* พบว่าการติดเชื้อแบคทีเรียส่งผลให้กุ้งก้ามกรามมีภูมิคุ้มกันลดลง

คำสำคัญ: การจำแนกชนิด *Lactococcus garvieae* *Macrobrachium rosenbergii* ความรุนแรง

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