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Characterization and Identification of Bacteriocin-Like Substances Producing Lactic Acid Bacteria from the Intestine of Freshwater Crayfish, *Cherax quadricarinatus*

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Abstract: Redclaw crayfish, *Cherax quadricarinatus* is a valuable species for aquaculture. However, bacterial disease is an important obstacle, thus, currently, conventional treatment using antibiotic is prohibited. In order to control bacterial disease, alternative treatment is crucial for crayfish aquaculture. One of the potential alternatives is bacteriocin or Bacteriocin-Like Substances (BLIS). Hence, the objective of this study is to identify the Lactic Acid Bacteria (LAB) which is able to produce bacteriocin-like substances from freshwater crayfish, *Cherax quadricarinatus*. The enumeration of LAB in *C. quadricarinatus* is 1.21×10^7 CFU/g. Fifty-four isolates were successfully isolated using MRS agar and screened for their antagonistic activity against *Aeromonas hydrophila*. Twenty-one or 38.89 % of LAB isolates exhibited antagonistic activity against *A. hydrophila* and therefore categorized as BLIS producing strains. The morphological and phenotypic characteristics revealed six different groups of LAB. The identities of six representative BLIS producing LAB (CQ19, CQ20, CQ21, CQ41, CQ42, and CQ53) were confirmed by 16S rRNA gene sequencing. These representatives were identified as *Enterococcus faecalis* CQ19, *E. faecalis* CQ20, *E. faecalis* CQ21, *E. faecalis* CQ41, *E. faecalis* CQ42 and *Lactobacilus plantarum* CQ53. These LABs were able to produce bacteriocin-like substances, illustrating their potential activity in disease control in crayfish aquaculture.

Keywords: Crayfish Aquaculture, Antibiotic Resistance, Alternative Prevention, Disease Control

Introduction

Redclaw crayfish, *Cherax quadricarinatus* is a valuable species, with high potential to be chosen as candidate of intensive aquaculture and ornamental culture (Belle and Yeo, 2010). They have been described as hardy species (Veselý *et al*., 2015), having wide range of tolerance to water parameters and stocking densities (Eaves and Ketterer, 1994; Medley, 1994). However, as crayfish aquaculture developed, bacterial diseases have become more obvious and varied (Tan and Owen, 2000). According to Longshaw (2011), occurrences of bacterial diseases are ordinary and potentially associates with opportunistic infection in farmed crayfish. There are two major bacteria pathogen which responsible for bacterial infection in Redclaw crayfish, namely *Vibrio mimicus* (Pandiyan *et al*., 2013) and *Coxiella cheraxi* (Saoud *et al*., 2013). Besides, Jiménez *et al*. (1997) reported Rickettsial infection caused 45% to 80% mortalities in Redclaw crayfish. Recently, mass mortalities in Redclaw crayfish was reported in a case of bacterial infections. According to Hayakijkosol *et al*. (2017), high mortality occurred in stage II crayfish craylings, the causative agent, reported to be *Aeromonas hydrophila* which was also most common freshwater pathogen worldwide (Zmyslowska *et al*., 2009).

Therefore, as a control for bacterial disease, antibiotic-incorporated feed as traditional measure is usually given to the farmed animals (Pridgeon and Klesius, 2012; Pandiyan *et al*., 2013). However, the application of chemicals for bacterial disease control has contributed to harmful effects to the environment and aquatic ecosystems (Rahman, 2014), damaging beneficial bacteria (Arthur *et al*., 2000); furthermore, application of antibiotics contributed to antimicrobial residue in aquaculture products and environment (Romero *et al*., 2012). Therefore, it is imperative to search for substituent of antibiotics.

Control of the problems and improve aquaculture yield has lead to the introduction of probiotics in aquaculture (Martínez *et al*., 2012). In general, probiotics exhibit promising results that benefit aquaculture in various ways (Pandiyan *et al*., 2013). One of the most common probiotics is known as lactic acid bacteria (LAB) and recognized as the most promising bacterial genera in aquaculture (Ringø,

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2018). Almost all bacteria species produced at least one type of bacteriocins (Riley and Wertz, 2002), as a defensive tool to protect themselves (Yang *et al*., 2014). Lactic acid bacteria are especially known for producing bacteriocins and other inhibitory compounds, such as acetaldehyde, hydrogen peroxide as well as diacetyl (Ringo and Gatesoupe, 1998; Gatesoupe, 1999). Bacteriocins, along with several compounds produced from bacteria were potentially used as inhibitor of bacterial pathogens in aquaculture (Tank *et al*., 2018). Bacteriocins are antimicrobial peptides which are synthesized by ribosome, and generally display high degree of target specificity against bacteria that are closely related strains and/or broad range antimicrobial activity (Cotter *et al*., 2013). Yang *et al*. (2014) described bacteriocins as a kind of antimicrobial peptides produced by bacteria, which can kill or inhibit closely related bacterial strains or non-related bacteria, but do not harm the bacteria themselves by specific immunity proteins.

Previously, researchers performed similar research on isolation of bacteriocin from animal source, even aquaculture animals. However, there is no research on isolation of bacteriocin from crayfish species. Thus, the objective of this study is to characterize and identify the LAB which are able to produce bacteriocin-like substances from intestine of freshwater crayfish, *Cherax quadricarinatus*.

Materials and Methods

Preparation and Collection of Sample

Farm cultured freshwater crayfish, *Cherax quadricarinatus* was collected from a local farm in Papar, Kota Kinabalu. Healthy crayfish samples were stored in dark and cold condition, by packing in ice box during the transfer from farm (Nair and Surendran 2005) to Fish Diseases Laboratory, University Malaysia Sabah.

Sample Processing

Intestine of crayfish sample was aseptically removed and weighted. Due to small amount of intestine from one individual, all intestines from all samples (n = 3) were pooled. Approximately, 0.3 g of intestine sample was collected and sterile saline (0.65% NaCl, pH 7.0) was added for homogenization with mortar and pestle.

Serial Dilution and Enumeration of Bacteria from Intestine

Subsequently, well-homogenized homogenate was transferred to individual test tube, diluted with sterile saline solution (0.65% NaCl, autoclaved at 121°C) eight times in serial 10-fold dilution to reduce cells density in the sample (Jalal *et al*., 2006). Aliquot of 0.1 mL from different dilutions were spread plated, in triplicates, on De Man, Rogosa and Sharpe (MRS) agar incubated at 30°C for 72 hours. The colony on the MRS agar plate was enumerated as colony forming unit (CFU) per gram of intestine sample (Lakshminarayanan *et al*., 2013).

Screening and Isolation of Bacteriocin-like substances producing LAB

Screening of potential bacteriocin-like substances (BLIS) producing strains was conducted by overlay assay (Hockett and Baltrus, 2017). The antibacterial activities of potential strains were examined against *Aeromonas hydrophila* ATCC in triplicate. Bacteria with different morphological appearance was selected and subculture on TSA for 24 hours at 30°C. Post-incubated TSA plate was overlaid with TSA soft agar (with *A. hydrophila*) before incubation at 37°C for 48-72 hours. After incubation, colonies that capable to form clearing zone was observed and subcultured on new MRS agar plate for purification (Nair and Surendran, 2005; El-Shafei *et al*., 2000). Pure culture of BLIS producing strains were preserved at glycerol stock (30%) before to be stored at -80°C.

Morphological Characterization

BLIS producing LAB were tested with Gram staining and colony morphology was followed the method described by Zhou and Li (2015) and Said *et al*. (2018). The colony morphology was explored according to the size, colour, shape, opacity, surface and elevation (Zhou and Li, 2015).

Biochemical Characterization

BLIS producing LABs were subjected to biochemical test using RapID NF Plus System (Ramel, San Diego, California, United State) (Ang and Lal, 2019) following manufacturer's instruction. RapID™ NS Plus panel were inoculated with bacteria and incubated at 37°C for 4 hours. Colour changes were observed and recorded. The isolates were grouped into phenotypic categories and the isolates with similar morphological and phenotypic characteristics were regarded as same species. One bacterial isolate was used as

representative for identification by 16S gene sequencing.

Molecular Identification of Selected Strains

DNA of selected bacteria isolate was then extracted using Wizard® Genomic DNA Purification Kit with minimal modification from Promega (2010) and Ang and Lal (2019). One mL of overnight bacteria culture was pelleted in centrifuge (13000 rpm for 2 minutes) and the supernatant was discarded. Bacteria cell was suspended in 480 µL 50 mM, pH 8.0 ethylenediaminetetraacetic acid (EDTA), followed by addition of 120 µL lysozyme (25mg/mL) and incubated at 37℃ for 45 minutes before centrifuge at 13000 rpm for 2 minutes. Supernatant was discarded and lysis of cell was conducted by adding 600 µL Nuclei Lysis solution. Well-mixed suspension was incubated at 80 ℃ for 5 minutes and left cooled to room temperature. Next, 3 µL of RNAse solution was added before incubated at 37 °C for 45 minutes. Subsequently, 200 µL of Protein Precipitation Solution was added. The well-mixed suspension was incubated on ice for 5 minutes before centrifuged at 13000 rpm for 3 minutes. About 600 µL of supernatant was transferred to new 1.5 mL tube. For precipitation of DNA, 600 µL of isopropanol was added. The mixture was mixed well and centrifuged for 2 minutes at 13000 rpm. The supernatant was removed before addition of 600 µL of ethanol (70%). The tube was centrifuged at 13000 rpm for 2 minutes. Ethanol was completely removed and DNA pellet was rehydrated with 100 µL rehydration solution before stored in -20°C. Subsequently, PCR amplification was performed using 13.6 µL of autoclaved distilled water, 5.0 µL of 5x colourless GoTaq® Flexi Buffer (Promega), 1.7 µL of $MgCl_2$ (25 mM, Promega), 0.5 µL dNTPs (10 mM, Promega), 1.0 µL forward primer and 1.0 µL reverse primer: 32F (25.1 nmol, N-Gene Research-Laboratories, New York, United States) and 1432R (24.1 nmol, N-Gene Research-Laboratories, New York, United States) respectively, 0.2 µL GoTaq® Flexi Polymerase (5 U/ µL, Promega) and 2.0 µL template DNA. The total volume of the reaction mixture was 50 µL. In this experiment, the forward primer used was 32F 5'-TCA GRW YGA ACG CTG GCG G-3' and reverse primer was 1432R 5'-CGA TTA CTA GCG ATT CCG RC-3', final concentration was 10 mM.

The PCR was conducted in Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific, United States) with pre-heating at 95 °C for 3 minutes prior 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 56 °C for 30 seconds, and extension at 72 °C for 30 seconds. The PCR was finalized by another extension period at 72 °C for 5 minutes. PCR product, upon completion of amplification, was purified using Invisorb® Fragment CleanUp (Stratec Molecular, Berlin, Germany). The purified PCR product was sent for sequencing at Biotechnology Research Institute, Universiti Malaysia Sabah. The sequencing result was analysed using Basic Local Alignment Search Tools (BLAST) analysis to confirm the identity of the bacterial strains (Levya *et al*., 2011).

Results

Isolation, enumeration and screening of BLIS producing Lactic Acid Bacteria

A total of fifty-four lactic acid bacteria were isolated from intestine of *Cherax quadricarinatus* and designated as CQ01 - CQ54. Bacteria isolates were serially diluted and enumerated, and the average colony count was 1.21 x 10^7 CFU/g. The screening result shows that 21 or 38.89 % from whole isolates exhibited antibacterial activity against *A. hydrophila* (Fig. 1; Tab. 1).

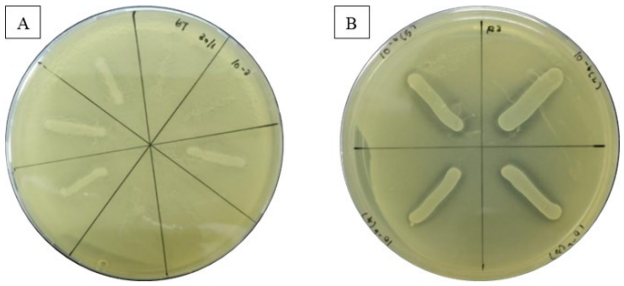

Fig. 1: Representative of antibacterial screening assay against *A.hydrophilla*. The antibacterial activity exhibited by the clearing zone at the vicinity of LAB isolates. A: Negative antibacterial activity; B: Positive antibacterial activity.

Morphological Characteristics of BLIS Producing LAB

Variable results observed in term of morphology of isolates (Tab. 2), all bacteria colonies shared similar characteristics in terms of surface, texture, and elevation; while most of them were in circular form, possessed shiny and smooth surface, and appear to be small colony (1-2mm). Only one strain of LAB samples having punctiform colony (<1 mm); while a few bacteria strains had medium size colony (3-4

mm). In addition, all colonies had opaque and white colour, with an exceptional isolate in translucent and pinpoint colony. All isolates were Gram positive bacteria with three types of cell shapes: rod, circular and oval (Tab. 2).

Tab. 1: Antibacterial screening result of 54 LAB isolates. "+": inhibition zone formed, "– ": no inhibition zone.

No	LAB Isolates	Inhibition zone	No.	LAB Isolates	Inhibition zone
1	CQ01	+	28	CQ28	–
2	CQ02	+	29	CQ29	–
3	CQ03	+	30	CQ30	–
4	CQ04	+	31	CQ31	–
5	CQ05	+	32	CQ32	–
6	CQ06	+	33	CQ33	–
7	CQ07	+	34	CQ34	–
8	CQ08	+	35	CQ35	–
9	CQ09	+	36	CQ36	–
10	CQ10	+	37	CQ37	–
11	CQ11	–	38	CQ38	–
12	CQ12	–	39	CQ39	–
13	CQ13	–	40	CQ40	–
14	CQ14	–	41	CQ41	+
15	CQ15	+	42	CQ42	+
16	CQ16	–	43	CQ43	–
17	CQ17	–	44	CQ44	–
18	CQ18	–	45	CQ45	+
19	CQ19	+	46	CQ46	+
20	CQ20	+	47	CQ47	+
21	CQ21	+	48	CQ48	+
22	CQ22	–	49	CQ49	–
23	CQ23	–	50	CQ50	–
24	CQ24	–	51	CQ51	–
25	CQ25	–	52	CQ52	–
26	CQ26	–	53	CQ53	+
27	CQ27	–	54	CQ54	–

Tab. 2: Colony morphology, cell shape and Gram characteristics of BLIS producing LAB isolates

BLIS Producing LAB	Colony Morphology							Cell Shape	Gram (– /+)
	Form	Size (mm)	Surface	Texture	Colour	Elevation	Margin		
CQ01	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ02	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ03	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ04	Circular	3	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ05	Circular	3.5	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ06	Circular	3	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ07	Circular	3	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ08	Circular	2.5	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ09	Circular	2.5	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ10	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ15	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ19	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ20	Circular	3	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ21	Circular	< 1	Shiny & Smooth	Moist	Translucent	Convex	Entire	Round	+
CQ41	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Oval	+
CQ42	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Round	+
CQ45	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ46	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ47	Circular	2.5	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ48	Circular	2	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+
CQ53	Circular	2.5	Shiny & Smooth	Moist	Opaque & White	Convex	Entire	Rod	+

Biochemical Characterizations

All BLIS producing LAB were positive for oxidase test. Based on the differences on the biochemical test results in Table. 3, there were three different phenotypes exhibited on five different biochemical tests. Thus, each isolate was categorized into phenotype 1, 2 and 3.

Molecular Identification

Out of 21 BLIS-producing strains, six of them with different morphological and phenotypic characteristics were selected for molecular identification, namely CQ19, CQ20, CQ21, CQ41, CQ42, and CQ53. Basic Local Alignment Search Tool (BLAST) results revealed that the identity of CQ19, CQ20, CQ21, CQ41, and CQ42 as *Enterococcus faecalis*, while CQ53 as *Lactobacilus plantarum* (Tab. 4).

Discussion

Gastrointestinal tract (GI) was selected as site of LAB collection, as it houses variety of lactic acid bacteria in aquatic organisms (Masduki *et al*., 2018). Lactic acid bacteria are part of the normal flora in GI tract in healthy animals (Nikoskelainen *et al*., 2001; Zapata and Lara-Flores, 2012). Ringo *et al*. (2018) described that bacteriocin originated and characterized from aquatic animals were scarce. According to Sahoo *et al.* (2006), bacterioncin-producing bacteria were mostly isolated from marine water. Cai *et al.* (1999) reported that the number of LAB from freshwater prawn was comparable with the current study. However, the composition of intestinal microbiota varied according to the surrounding environment; and can be modified by supplementing diets (Rengpipat *et al*., 2000).

Tab. 3: Biochemical Characteristics of BLIS producing LAB isolates. " + ": positive result, " - ": negative result.

BLIS Producing LAB	Biochemical characteristics							Phenotype
	Positive	Negative	ADH*	ONPG*	PRO*	PYR*	TRY*	
CQ01			–	–	+	–	–	1
CQ02			–	–	+	–	–	1
CQ03			–	–	+	–	–	1
CQ04			–	–	+	–	–	1
CQ05			–	–	+	–	–	1
CQ06	-Aliphatic thiol utilization		–	–	+	–	–	1
CQ07	-ρ-Nitrophenyl-phosphoester	-Triglyceride hydrolysis	–	–	+	–	–	1
CQ08	-ρ-Nitrophenyl-N-acetyl-β,D-glucosaminide hydrolysis	-urease enzyme	–	–	+	–	–	1
CQ09	-ρ-Nitrophenyl-α-D-glucoside hydrolysis	-γ-Glutamyl β-naphthylamide hydrolysis	–	–	+	–	–	1
CQ10	- ρ-Nitrophenyl-β-D-glucoside hydrolysis	-N-Benzyl-arginine-β-naphthylamide hydrolysis	–	–	+	–	–	1
CQ15	-D-glucoside hydrolysis	-tryptophane utilization	–	–	+	–	–	1
CQ19	-glucose utilization	-nitrate utilization	–	+	–	+	–	2
CQ20			+	+	–	+	+	3
CQ21			+	+	–	+	+	3
CQ41			+	+	–	+	+	3
CQ42			+	+	–	+	+	3
CQ45			–	–	+	–	–	1
CQ46			–	–	+	–	–	1
CQ47			–	–	+	–	–	1
CQ48			–	–	+	–	–	1
CQ53			–	–	+	–	–	1

*ADH = Arginine hydrolysis; ONPG = ρ-Nitrophenyl-β,D-glucoside hydrolysis; PRO = Proline-β-napthylamide hydrolysis; PYR = Pyrrolidine-β-napthylamide hydrolysis; TRY = Tryptophane-β-napthylamide hydrolysis; + = positive; – = negative

Tab. 4: Highest sequence homology from BLAST results of six selected isolates.

Isolates	Homolog	E value	Homology (%)	Species Name
CQ19	*Enterococcus faecalis* strain B8.1.1 16S ribosomal RNA gene, partial sequence	0.00	99.43	*Enterococcus faecalis*
CQ20	*Enterococcus faecalis* strain P190052 16S ribosomal RNA gene, partial sequence	0.00	99.73	*Enterococcus faecalis*
CQ21	*Enterococcus faecalis* strain 123 16S ribosomal RNA gene, partial sequence	0.00	96.98	*Enterococcus faecalis*
CQ41	*Enterococcus faecalis* strain P190052 16S ribosomal RNA gene, partial sequence	0.00	98.88	*Enterococcus faecalis*
CQ42	*Enterococcus faecalis* strain P190052 16S ribosomal RNA gene, partial sequence	0.00	99.19	*Enterococcus faecalis*
CQ53	*Lactobacillus plantarum* strain YK-9 16S ribosomal RNA gene, partial sequence	0.00	99.05	*Lactobacilus plantarum*

In this study, the inhibition produced by LAB isolated from *C. quadricarinatus* suggest the potential of LAB isolates to be utilized in aquaculture as alternative for antibiotics. It is regarded safe for human consumption, since the proteinaceous bacteriocin fragment were easily degraded with the present of proteolytic enzyme in host (Zacharof and Lovitt, 2012). Thus, this reduced the interaction with target strains and the development of resistance (Ringo *et al*., 2018). Therefore, treatment of farmed aquatic animals with bacteriocin instead of commercial antibiotics could be more attractive and gain more trust from consumer (Ringo *et al*., 2018).

The LAB isolates in this this study showed similar morphological characteristics to isolates observed by Sameen *et al*. (2010) and Sulmiyati *et al*. (2019). Besides, variations observed in this study in terms of colony size and colour, all colonies were small with the exception of CQ21 that was pinpoint and translucent. In addition, cell morphology in this study was also corroborated with the appearances of lactic acid bacteria summarized in Farzanfar (2006), Ismail *et al*. (2018), Masduki *et al*. (2018) and Ringø *et al*. (2018). According to Rather *et al.* (2017), gram positive bacteria account for the biggest portion of the bacteriocins producing bacteria, together with small part of gram negative bacteria. The biochemical test revealed that there were three different phenotypes of LAB in this study. These differences in phenotypic characteristics may suggest the isolates of this study belong to different species of LAB. Based on these biochemical and morphological characteristics, there are six presumptive LAB species isolated in this study. For all the aforementioned reasons, six BLIS producing lactic acid bacteria in this study belonged to genera of *Enterococcus* and *Lactobacillus*. This shows that these two genera are common LAB species in intestinal tract of animals. *Lactobacillus* species was identified as one of the LAB in intestine of shrimp (Ang and Lal, 2019). This finding is comparable to the finding of Nursyirwani et al. (2017) where LAB isolated from intestinal tract of tiger grouper (*Epinephelus fuscogutattus*) was *Enterococcus* spp. Interestingly, some species exhibited different morphological and biochemical characteristics. However, the role of these different strain of *E. faecalis* in this study is still unknown. This study found that the composition of intestinal LAB isolates of Redclaw crayfish are 83% of *E. faecalis* and 17% of *L. plantarum*. Both genera were previously reported as common species of LAB in gut of aquatic animals (Allameh *et al*., 2014; Čanak *et al*., 2018), however, the species composition of LAB in gut is still unknown.

According to Ghanbari *et al.* (2013), *L. plantarum* are able to produce bacteriocin like substances that inhibiting pathogens including *A. hydrophila*, supporting the current study. Besides, Messi *et al*. (2001) stated that bacteriocin of *L. plantarum* was active against *A. hydrophila*, and was reported that is able to inhibit following pathogens: *E. coli*, *Listeria spp*., *Salmonella spp*., *S. aureus*, *V. anguillarum* and *B. cereus*. Previously, *L. plantarum* has been reported in the gut of Persian sturgeon (Ghanbari *et al*., 2009) and even been isolated from Turkish dairy products (Aslim *et al*., 2005). *E. faecalis* belongs to large genus of LAB. In fact, enterococci are commensal bacteria in general, and their major function in this aspect is to facilitate the digestion and other gut metabolic pathway (Byappanahalli *et al*., 2012). The

morphological observation of this isolate in this study showed that it was spherical or ovoid cell arranged in pair or chain and tested Gram positive, which is supported by the core morphological feature of the member in Enterococcus genus, as describe in Lebreton *et al.* (2014). This species has been reported in the marine environment, and it is a vital pathogen in aquaculture, it has been noted poses serious impacts in commercial aquaculture species worldwide. In addition, Rather *et al.* (2017) revealed that *E. faecalis* (subsp. liquifaciens) is one of the producers of class IV Gram positive bacteriocins. Our findings suggested that *E. faecalis* contained potential to be used in aquaculture.

Conclusion

Two species of lactic acid bacteria were successfully identified from intestine of freshwater crayfish, *C. quadricatinatus* were able to produce bacteriocin-like substances. The isolated bacteriocin-like substances producing lactic acid bacteria from Redclaw crayfish might help other aquaculture animals in disease management. Nevertheless, further research such as optimization of bacteriocin-like substances producing need to be conducted to achieve maximum effect of a bacteriocin-like substance produced before it can be applied in aquaculture as an alternative of antibiotics.

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