Antibacterial Activities of *Bdellovibrio* and like Organisms in Aquaculture

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1 Introduction

Aquaculture is the cultivation of aquatic living organisms, especially fish, shellfish, crustaceans, molluscs and seaweed in natural or controlled freshwater or marine environments. With the development of economy and the improvement of living standards of growing population, demand for aquatic products in the world is rapidly rising. In the past few decades, aquaculture has increasingly contributed to the food production, supplying raw materials for industrial and pharmaceutical uses, as well as for ornamental fish trade. While continuing to rely on traditional fishing,

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aquaculture industry has been vigorously developed to make up for the lack of supply in the consumer market. Hence, it has quickly become one of the fastest growing and most auspicious industries for providing animal super molecules and food security to the planet population (Le 2010; De et al. 2014). Taking China as an example, its national aquatic product output was increased from 59,076,800 metric tons in 2012 to 690,012,500 metric tons in 2016, an increment of 16.82% (FSF 2018). It is expected that the growth of aquaculture industry will continue at an even faster pace in the coming future.

However, production of fish, shellfish and seafood is often disrupted by environmental pollution, resource allocation and unpredictable mortalities that are the results of negative interactions between aquatic organisms and pathogens (Cabello 2006). Disease outbreaks in aquaculture are more and more common, becoming a severe problem which affects both the economic development and the socio-economic status of the people involved in many countries. In fact, there are actually hundreds of diseases that can affect farmed organisms. A majority of them are caused by bacteria like *Aeromonas (Ae.) hydrophila* (Irianto and Austin 2002), *Bacillus* (*Ba.*) *cereus* (Liu et al. 2016), *Edwardsiella* (*Ed.*) *tarda* (Irianto and Austin 2002), *Flexibacter columnaris* (Wakabayashi 1991), *Pseudomonas* (*Ps.*) *fluorescens* (Wang 2010; Austin and Austin 2016; Zhang et al. (2009b), *Ps. aeruginosa* (Cai et al. 2009), various species of *Vibrio* (*V.*) (Cheng et al. 2008; Al-Sunaiher et al. 2010), to name just a few.

In freshwater aquaculture, *Aeromonas* is considered a major problem (Zmyslowska et al. 2009; Cao et al. 2010). In mariculture, vibriosis, as caused by a number of *Vibrio*, like *V. harveyi, V. parahaemolyticus, V. alginolyticus, V.* (*Listonella, Lis.*) *anguillarum,* and *V. vulnificus*, is a major threat (Chatterjee and Haldar 2012). Early Mortality Syndrome (EMS), also known as Acute Hepatopancreatic Necrosis Disease (AHPND), is a newly emerged disease in penaeid shrimp [*Litopenaeus* (*Lit.*) *vannamei*] aquaculture, which is caused by a unique strain of *V. parahaemolyticus* carrying a plasmid that contains toxin genes homologous to *Photorhabdus* insect-related toxins (Tran et al. 2013; De Schryver et al. 2014). Its mortality rates can reach as high as 100% within a few days after occurrence of the disease (Wang et al. 2018). In addition to bacterial diseases, there are also viral diseases such as White Spot syndrome (as caused by white spot syndrome virus, WSSV) and Taura syndrome (as caused by Taura syndrome virus, TSV) in shrimp (Bondad-Reantaso et al. 2005) and parasitic diseases (such as caused by protozoan ciliates, *Ichthyophthirius* sp., *Trichodina* sp.) (Bondad-Reantaso et al. 2005). Most if not all of them, regardless of bacterial or viral nature, are conditional pathogens that cause infections or disease outbreaks when environmental conditions are deteriorated (and thus their numbers are high) and/or cultured organisms are under stress (De Schryver and Vadstein 2014). Therefore, elimination of pathogens or potential pathogens, or a reduction of their numbers, would help reduce the chances of disease outbreaks.

Currently, three types of strategies are being deployed to control pathogens and to protect farmed organisms from diseases, viz., chemical, physical and biological means.

Chemically: to control pathogens/diseases, aquaculture entities frequently use chemicals or antibiotics to combat infections (Cabello 2006). Various studies have already pointed out the negative impacts, in that the use of chemicals and antibiotics in aquaculture could result "in the emergence of antibiotic-resistant bacteria in aquaculture environments, in the increase of antibiotic resistance in fish pathogens, in the transfer of these resistance determinants to bacteria of land animals and to human pathogens, and in alterations of the bacterial flora both in sediments and in the water column" (Cabello 2006). Growing global concerns about chemical and antibiotic negative effects makes it necessary to seek environmentally friendly alternatives for a sustainable aquaculture production.

Physically: UV and Ozone (Summerfelt 2003) and filtration (Wold et al. 2014) techniques are being used to treat water and to reduce microorganisms in some sections of aquaculture, in shrimp larviculture in particular.

Biologically: probiotics, prebiotics and their combination (synbiotics), bacteriophages and nonviable bacterial products are increasingly being employed to control microbes and to prevent diseases in aquaculture as well as to improve water quality (Pérez-Sánchez et al. 2018).

As a potentially new type of probiotics, the predatory bacteria *Bdellovbrio* and like organisms (BALOs) are increasingly being applied in aquaculture, especially in China. Here in this chapter, we will review relatively high quality documented studies to assess BALOs antibacterial activities related to aquaculture and to evaluate their application potentials in aquaculture.

2 Probiotics in Aquaculture

Probiotics are delineated as live, dead or components of microbial cells which confer health benefits, better growth performances, less stress responses or better general vigour on the host when administered in an adequate amount (Gatesoupe 1999).

The concept of probiotics in aquaculture is relatively new, but their applications have been gaining popularity due to the demand for a sustainable and environmentally friendly aquaculture (Gatesoupe 1999; Newaj-Fyzul et al. 2014).

Up to now, probiotics used in aquaculture included yeasts like *Debaryomyces* sp., *Phaffia* sp. and *Saccharomyces cerevisiae* (Irianto and Austin 2002), various *Bacillus* species (Del'Duca et al. 2013), denitrifying bacteria (Wang et al. 2018), photosynthetic bacteria like *Rhodobacter sphaeroides* (Wang 2011), as well as lactic acid bacteria like *Lactobacillus* (Aguilar-Macias et al. 2010), *Enterococcus faecium* (Swain et al. 2009), and *Carnobacterium* (Kim and Austin 2006). Even some specific strains of the following genera have also been evaluated as probiotics due to their potentially beneficial natures: *Ae. hydrophila* A3–51 (Irianto and Austin 2002), *Ps. fluorescens* (Hai et al. 2009), *Shewanella* (*Sh.*) sp. (García De La Banda et al. 2012; Tapia-Paniagua et al. 2012; Jiang et al. 2013), and even *V. fluvialis* (Alavandi et al. 2004) and *Vibrio* spp. (Thompson et al. 2010).

BALOs had been proposed as a bio-agent around 1990s in China (Qin 1987; Yang and Huang 1997) and are gaining momentums from the start of this century (Yang et al. 2004; Li et al. 2017).

3 *Bdellovibrio* and like Organisms (BALOs)

BALOs are a group of small (0.25 μm wide and up to 2 μm long), rapidly motile, aerobic, Gram-negative and obligate predatory bacteria that are capable of invading/surrounding other bacteria for growth, reproduction, and survival (Jurkevitch and Ramati 2000; Rotem et al. 2014; Stolp and Starr 1963). The first observation of this tiny and rapidly moving microorganism was made by Stolp and Petzold (1962).

Taxonomically, Koval et al. (2015) reclassified the then-existing BALOs of class Delta-proteobacteria into four families, i.e., (I) family *Bdellovibrionaceae* with *Bdellovibrio* (*Bd.*) *bacteriovorus* as type species and *Bd. exovorus* as another identified species, (II) family *Halobacteriovoraceae* with *Halobacteriovorax* (*Hal.*) *marinus* as type species and *Hal. litoralis* as another identified species, (III) family *Bacteriovoracaceae* with *Bacteriovorax* (*Bact.*) *stolpii* as type species, and (IV) family *Peredibacteraceae* with *Peredibacter starrii* as type species. In the same year (2015), McCauley et al. (2015) proposed within the order *Bdellovibrionales* a new family *Pseudobacteriovoracaceae* with a new genus *Pseudobacteriovorax* (*Pseudobacteriovorax antillogorgiicola* RKEM611^T as the type strain). Then in 2017, with more comprehensive and in-depth research, Hahn et al. (2017) reclassified BALOs taxonomy, with the establishment of a new order *Bacteriovoracales* to encompass families *Bacteriovoracaceae* (Davidov and Jurkevitch 2004) (genera *Bacteriovorax* and *Peredibacter*), and *Halobacteriovoraceae* (Koval et al. 2015), with *Bacteriovorax* as the type genus; an emendation of the existing order *Bdellovibrionales* (Garrity et al. 2005) to only include genera *Bdellovibrio*, *Micarvibrio*, and *Vampirivibrio*, as well as other unclassified BALOs, with *Bdellovibrio* as the type genus; a reclassification of the family *Pseudobacteriovoracaceae* in the order *Oligoflexiales*. All these three orders, viz., *Bdellovibrionales*, *Bacteriovoracales* and *Oligoflexiales*, are under the class *Oligoflexia* (Nakai et al. 2014). Thus, BALOs belong no more to the class *Delta-* or *Alpha- proteobacteria*.

Reproductionally, *Bd. bacteriovorus* is the best studied member of all (Sockett and Lambert 2004). Its fast swimming attack-phase cells interact with their preys, attaching to the prey cells, penetrating prey cell wall and stay in their periplasm (which is called periplasmic predation) (Pasternak et al. 2014). This stage is called growth (or periplasmic) stage. There, it grows and multiplies, ending in the lysis of prey cells and the release of bdellovibrio progenies (Abram et al. 1974; Rotem et al. 2014). For more details, please consult the Chapter by Jurkevitch on BALOs in wastewater.

Depending on the environmental conditions and prey hosts, completing a whole life cycle takes roughly 3–4 h (Nunez et al. 2005). Further discussion on

environmental factors and their impacts on predation is available in the chapter by Mitchell. Because of this unique prey-attack characteristic, BALOs have been proposed as living alternatives to chemical and antibacterial agents in environment and public health (Sockett and Lambert 2004; Rotem et al. 2014), or as a bio-agent for use to control pathogens in mariculture (Yang et al. 2004).

3.1 Natural Existence of BALOs in Aquatic/Aquaculture Habitats and the Guts of Cultured Organisms

BALOs are widely distributed in nature (Fry and Staples 1976; Williams et al. 1995; Cai et al. 2008).

To examine BALOs natural existence in freshwater habitat, Shi et al. (1987) collected water (or mud) samples from sea, lakes, rivers and ponds from 258 places in 31 cities and counties across Anhui, Jiangsu, Shandong provinces and Beijing from November 1979 through April 1985. They employed 5 hosts for the detection of BALOs in each sample, viz., *V. cholera* biotype El Tor, *Shigella* (*Shi.*) *flexneri*, *V. parahaemolyticus*, and *Escherichia* (*Es.*) *coli*. Out of totally 325 samples, 254 samples showed the presence of BALOs, amounting to a positive rate of 78.15%. Their densities ranged from 1 plaque forming unit (PFU) per mL (or g of mud) to 5.88×10^3 PFU per mL (or g of mud). Unfortunately, the authors did not correlate the positive rates with months or seasons so as to rule out the temperature effect, as it could impact BALOs presence in nature (Sutton and Besant 1994). Yu et al. (1994) then conducted a survey in Spring (March to April) of 1993 on five major rivers in Chengdu city, China. They used the following host strains for each sample, viz., *Es. coli* 8099, *Ps. aeruginisa* 10123, *Shi. flexneri* F2a.1180, *Salmonella* (*Sa.*) *typhimurium*, *Ba. subtilis* 8017, *Ba. cereus* 4001, *Staphylococcus* (*St.*) *aureus* 6538, and found BALOs presence in all five rivers with an average content of 2.1×10^4 PFU mL^{-1}, ranging from 4.0×10^2 PFU mL^{-1} to 1.0×10^6 PFU mL^{-1}. On the basis of plaque forming characteristics, the authors isolated 5 strains of BALOs and found all 5 strains could lyse *Es. coli* 8099, *Shi. flexneri* F2a.1180, *Sa. typhimurium*, 4 strains could lyse *Ps. aeruginosa* 10123 and Gram-positive *Ba. cereus* 4001, and 3 strains could lyse Gram-positive *St. aureus* 6538. These studies not only demonstrated the natural existence of BALOs in freshwater environments, even at relatively high densities in some habitats, but also revealed their different lytic characteristics.

With respect to marine habitat, Taylor et al. (1974) had recovered 13 strains of *Bdellovibrio* from sea water off the coast of Oahu, Hawaii and the abundance of *Bdellovibrio* was 121–194 PFU per liter of sea water. Williams et al. (1995) recovered *Bdellovibrio* from submerged surfaces and other aquatic habitats of Chesapeake Bay, i.e., water and sediment, oyster shell surface biofilms, zooplankton, and plants. More recently, Li et al. (2011) isolated two strains of BALOs, viz., BDH12 and BDHSH06, from sediment of Daya bay in Shenzhen of China using *Sh.*

putrefaciens strain 12 and *V. parahaemolyticus* strain SH06 as prey, respectively. These two strains may form a new genus within the family *Bacteriovoracaceae* on the basis of partial 16S rDNA sequence analysis.

Apart from naturally existing waters, BALOs are also widely distributed in various man-made waters, like aquaculture environments. For instance, Schoeffield and Williams (1990) recovered *Bdellovibrio* from the water of a brackish tidal pond and also from an aquarium saltwater tank using *V. parahaemolyticus* P-5 as host organism. Yang and Huang (1997) isolated 44 strains of BALOs from marine shrimp farms. Their further studies showed that these 44 different strains had different prey ranges. While most of them could lyse Gram-negative bacteria like *V. cholerae* non-01, *V. harveyii*, *V. parahaemolyticus*, *V. alginolyticus*, *V. fluvialis*, *V.* (*Lis.*) *anguillarum*, *Es. coli*, *Ps. aeruginosa*, some could even lyse Gram-positive bacteria *Ba. subtilis* and *St. aureus*. Chu and Zhu (2010) utilized *Ae. hydrophila* J-1 as prey organism and isolated 14 BALO strains from cultured cyprinoid fish ponds. Among them, strain BdC-1 could lyse 23 Gram-negative bacteria comprising three genera of fish pathogens (i.e., *Ae. hydrophipla*, *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyii* and *Ed. tarta*) and one strain of *Es. coli*, but could not lyse *Ba. subtilis* and *St. aureus*. To further explore BALOs natural existence and diversities, Wen et al. (2009) used two PCR-based methods to type saltwater BALOs in shrimp mariculture systems. The number of culturable BALOs that lysed *V. alginolyticus* was found to be in the range of $10–10^3$ PFU mL^{-1} in the surface water samples using double-layer agar technique. Among 130 BALOs they isolated, five and four phylotypes were revealed by denaturing gradient gel electrophoresis targeting the 16S rDNA V3 region and amplified rDNA restriction analysis of the *Bacteriovoracaceae* specific 16S rDNA fragment, respectively. Their phylogenetic analysis further showed that all of the representative isolates were identified as *Bacteriovorax* spp., but separated into four different clusters in the family *Bacteriovoracaceae*. This finding demonstrated that the relatively large number of saltwater BALOs with diverse phylotypes was naturally present in shrimp mariculture environments and they might well play an important role in shrimp farming ecosystem.

Apart from their existence in various waters, BALOs are also naturally present on aquatic (wild or cultured) organisms or in their guts. Using double-agar-overlay technique with *V. parahaemolyticus* P-5 as host, Kelley and Williams (1992) recovered BALOs from the gills of all 31 samples of blue crab (*Callinectus sapidus*) from different geographical regions in Chesapeake Bay and seasons (4 seasons). Zhang et al. (2009c) recovered *Bdellovibrio* sp. Bdm4 from the gut of Eel (*Anguilla* spp.) using *Ae. hydrophila* as prey. Cao et al. (2007) isolated *Bdellovibrio* sp. BDF-H16 from the gut of gibel carp [*Carassius* (*Ca.*) *auratus gibelio*] using *Ae. sobria* as host. They later also isolated *Bd. bacteriovorus* strain F16 from sturgeon [*Acipenser* (*Ac.*) *baerii*] gut using a sturgeon-pathogenic *Ae. hydrophila* as prey (Cao et al. 2012). More recently, Han et al. (2015) used molecular typing techniques to study BALOs diversities in the intestine of spiny sea cucumber [*Apostichopus* (*Ap.*) *japonicas*] and found *Bdellovibrio* and *Bacteriovorax* were naturally present in the guts. On the basis of phylogenetic features, they suggested

that potentially five new BALOs species could be proposed, but no further identification has yet been done.

Until now, documented findings on the natural existence of BALOs in the guts of various aquatic organisms are relatively few. The reason for this, apart from very few studies performed on the various organisms in aquaculture, could be due to the combination of the following three factors, viz., the methods used for their studies, their relatively rarities in the guts and various environmental factors (see Sect. 3.2). Traditionally, we tend to use the culture dependent method, i.e., double-layer plating, to isolate and study BALOs, rather than more sensitive modern molecular methods. For the double-layer plating method, the number of BALOs in the guts needs to be sufficiently high enough to be grown, even when an appropriate/lysable host is used. Once they are below certain numbers, double-layer plating method might not be able to recover them as other dominant bacteria could well overgrow in the culture. This argument is supported by the finding of Zeng et al. (2017), who followed pacific white shrimp (*Lit. vannamei*) from larval stage (15 days post-hatching) to adult stage (75 days post-hatching) in order to investigate the intestinal microbiota at different culture stages. By high throughput sequencing that targeted the V4 region of 16S rRNA gene, they found that the abundance of *Bdellovibrio* in all shrimp intestine samples was relatively rare, with only 0.002%, while other microbes were much higher, i.e., *Candidatus_Xiphinematobacter* and *Propionigenium*, both 3.4%; *Synechococcus*, 2.7%; *Shewanella*, 1.3%; *Cetobacterium*, 1.1%; *Bacillus*, 0.9%; *Robiginitalea*, 0.7%; *Fusibacter*, 0.5%; *Arcobacter*, 0.5% and *Lactobacillus*, 0.04%.

The following two studies not only further confirm the natural existing of BALOs in shrimp guts, but surprisingly demonstrate a beneficial link between their abundance in guts and shrimp health or growth. The first study was done by Yang et al. (2016) who used Illumina sequencing to investigate the intestinal bacterial community composition of healthy and diseased juvenile shrimp *(Lit. vannamei*). They found that "the relative abundances of *Planococcaceae* and *Bacteriovoracaceae* families significantly decreased, while that of *Vibrionaceae* remarkably increased in diseased juvenile shrimp digestive tract in relation to healthy one". This indicated that higher abundances of BALOs in guts are linked with better shrimp health. The second study was performed by Xiong et al. (2017), who also employed high throughput sequencing to study the underlying ecological processes of gut microbiota among cohabitating retarded (slow grow), overgrown (fast grow) and normal (normal grow) shrimp (*Lit. vannamei*). They discovered that *Bdellovibrionaceae* was present in all shrimp groups, but highest in the overgrown ones. This means that higher abundances of BALOs in guts are linked to higher shrimp growth rates. The findings of these two studies are very similar to what we have already learnt in human as Iebba et al. (2013) revealed a higher prevalence and abundance of *Bd. bacteriovorus* in the human gut of healthy subjects, implying that BALOs do contribute to the health of various hosts, regardless of reared organisms or human.

3.2 *Some Environmental Factors that Affect BALOs Natural Existence*

As to the environmental factors that affect BALOs presence and/or quantities, and in turn affect their recovery rates in the laboratory, previous studies have revealed that BALOs diversity and abundance in aquatic and aquaculture environments depend on the factors such as water temperature, pH, salinity and seasons, types of habitats (like water surface, water column, sediment and body parts of aquatic animals), and many more. Fry and Staples (1976) noted the positive correlation between the quality of river water and the number of bdellovibrios, viz., bdellovibrios were present in all liquid phases of sewage river sediments and polluted river waters but not in some unpolluted river waters. Seasonal influence on the abundance of BALO recovery was noted by Sutton and Besant (1994), in that the abundance of bdellovibrios was correlated with water temperature and status of habitats during particular seasons of the year. They also found the differences in the vertical distribution of bdellovibrios in the water column among three different tropical marine habitats of the Great Barrier Reef in Australia. They revealed that the number of bdellovibrios was more in sub-surface water than bottom waters in summer, but the reverse occurred in winter while in midwater its presence was the least in all seasons of the year. Interestingly, an opposite finding was reported by Williams and Falkler (1984) who found no significant differences between the abundance of bdellovibrios recovered from several depths of the water column at a site in the Miles River. This discrepancy might be due to the presence of water stratification in Great Barrier Reef and not in Miles River.

Some studies revealed that BALOs are surface-associated organisms and their recovery numbers are several 100-fold higher from the surface water microlayer than from subsurface waters (Williams 1987). In fact, it has been suggested that bdellovibrios prefer to associate with surfaces as they could be recovered from the shell of oysters as well as the epibiota on other surfaces in the aquatic environment (Kelley et al. 1997; Williams et al. 1995). More recently, Zhang et al. (2016) determined the diversity of microorganism communities and the relationship between microbial communities and hosts in *Lit. vannamei* aquaculture water and environmental factors at Chenghu Lake, Kunshan City, China. They found that the abundance of the pathogenic bacterial genus *Flavobacterium* and probiotic bacterial genus *Bdellovibrio* correlated positively with pH, total nitrogen and chemical oxygen demand (COD), and negatively with water temperature and ammonia nitrogen (NH_3-N). This means that BALOs would be more in organic rich environments, a result that is consistent with the finding of Fry and Staples (1976).

3.3 Prey Ranges of BALOs for Aquaculture Purposes

Various studies, and our own experience, have demonstrated that different strains of BALOs possess very different lytic capabilities against their bacterial hosts, and thus showing very different ranges of prey spectrum (Table 1). Some have very wide prey ranges, covering many Gram-negative bacteria, and even some Gram-positive bacteria, while others have very narrow ranges, covering only few species or strains. For example, Kongrueng et al. (2017) showed that *Bacteriovorax* sp. isolate NBV3 displayed a widest prey range (13 out of 14 strains tested, ca. 92.86% lysis rate), lysing all 5 (AHPND)-causing strains of *V. parahaemolyticus* (viz., EMS_1S_2, VP12, 7.2 L3, PeP_{16}, 6.1 L3), 2 clinical Vp strains (PSU5666, PSU5668), 2 environmental Vp strains (PSU5147, PSU5150), *Es. coli, V. alginolyticus, V. cholera* and *V. vulnificus*, but could not lyse *St. aureus*. Isolate MBV6 had the narrowest prey spectrum (5 out of 14 strains, ca. 35.71% lysis rate). Meanwhile, isolates BV-A and MBV5 did not have the widest prey spectrums, but they could lyse Gram-positive *St. aureus*. Furthermore, Chu and Zhu (2010) also showed that out of 14 BALOs they isolated in total, an isolate, designated as *Bdellovibrio* BdC-1 (It is more appropriate to use the term BALO here, as molecular identifications were not performed), formed the largest plaque on the double-layer plates. This isolate had a widest prey range and could attack 24 out of 26 prey strains tested (i.e., 92.31% preys tested could be lysed). It lysed all strains of Gram-negative fish pathogens, viz., *Ae. hydrophila* J-1, Y-1, S-1, 1292, TPS30, HAE-1, X-1, NL-1, GML, BJ, AhS-2, AN-1, BX-50, MF-1, SF911212D, A7, LS-4, M13, W-1*; Ed. tarta* M1*; V. alginolyticus* HY-1*; V. harveyi* BK*; V. parahaemolyticus* HY-2, but not Gram-positive bacteria *Ba. subtilis* CGMCC1.884 and *St. aureus* CGMCC1.89 (Chinese General Microbiological Culture Collections, Beijing, China). Huang et al. (2010) also showed that *Bdellovibrio* strain 506 and strain 512 (again, the term BALO would be more appropriate here as molecular identifications were not performed), which were isolated from seawater, could attack 29 (93.55% lysis rate) and 24 (77.42% lysis rate) out of 31 pathogenic vibrios strains tested. At the low end, Cai et al. (2008) isolated 4 strains of BALOs, viz., BDW01, BDW02, BDW03 and BDW04, and found that they lysed only 15 (36.6%), 16 (39.0%), 27 (65.8%), 26 (63.4%) out of 41 vibrio strains tested, correspondingly. Clearly, these data illustrate the strain specificities in the lysis of various preys. Finding whether or not BALOs strain lysis specificities have any associations with their origins or taxonomic classification, requires much more work.

Another interesting point we noted is that if different species of hosts are used for isolation, BALOs thus obtained may display lysis preference towards that type of species. For example, Li et al. (2011) employed *Sh. putrefaciens* strain 12 and *V. parahaemolyticus* strain SH06 for isolation and obtained BDH12 and BDHSH06, respectively. Their lysis experiments showed that though both BALOs shared 68.4% (39 out of total 57 strains) of the strains as their common preys, BDHSH06 demonstrated a higher prey (36 out of 39 strains, 92.3% lysis rate) toward marine vibrios, while BDH12 showed a higher predatory ability (16 out of 18 strains,

Table 1 A list of various BALOs for aquaculture purposes and their prey ranges

BALOs strains	Sources	Prey host	Gram nature	Bacteria that are susceptible to relevant BALOs	References
BD04	Freshwater crab pond sediments	*Ae. hydrophila* B2	Negative	*Ae. hydrophila* B2; *Ed. tarda* B1; *Es. coli* C600	Zhou et al. (2011)
			Positive	*St. aureus*	
BdC-1	Freshwater fish ponds	*Ae. hydrophila* J-1	Negative	*Ae. hydrophila* J-1, Y-1, S-1, 1292, TPS30, HAE-1,X-1, NL-1, GML, BJ, AhS-2, AN-1, BX-50, MF-1, SF911212D, A7, LS-4, M13, W-1*; Ed. tarta* M1*; Es. coli* DH5α; *V. alginolyticus* HY-1*; V. harveyi* BK*; V. parahaemolyticus* HY-2	Chu and Zhu (2010)
Bacteriovorax sp.	Shrimp farm saltwater and sediments	4x AHPND causing strains (PSU5429, PSU5499, PSU5562, PSU5579)	Negative	*Es. coli; V. alginolyticus; V. cholerae; V. parahaemolyticus* (AHPND causing strains: PSU5429, PSU5499, PSU5562, PSU5579, EMS_1S_2, VP12, 7.2L3, PeP_{16}, 6.1L3*;* clinical strains: PSU5666, PSU5668 and environmental strains: PSU5147, PSU5150), *V. vulnificus*	Kongrueng et al. (2017)
			Positive	*St. aureus*	
Bd19-9899, Bd20-9899, Bd25-9899	Freshwater fish ponds and other waters	*Ae. hydrophila* SC9626, *Ae. punctata* 58-20-9, *Ps. fluorescens 56-12-10, V.* (*Lis.*) *anguillarum* E3-11	Negative	*Ae. hydrophila* SC9626, *Ae. punctata* 58-20-9, *Ps. fluorescens* 56-12-10*, Ps. stutzeri* 9899, *V. (Lis.) anguillarum* E3-11	Ma et al. (1999)
Bdh5221	Shrimp pond seawater	*Ps. stutzeri*	Negative	*Ae. hydrophila; Ed. tarda; Es. coli; Ps. fluorescens; V. alginolyticus; V.* (*Lis.*) *anguillarum; V. harveyi; V. parahaemolyticus*	Xie et al. (2007)
			Positive	*Ba. subtilis*; *St. aureus, Sarcina* sp.	

4.2, 5.1, 3N.3	Coastal seawater and sediments	*Es. coli* 21, AB90054	Negative	*Ae. hydrophila* 1.927, Sc-96-24, Ah9802120388; *Ps. fluorescens* ATCC10646; *Ps. putrefaciens* 0397; *V. alginolyticus* 1833; *V. cholerae* B0165; *V. harveyi* V-1-3120, B0150; *V.* (*Lis.*) *anguillarum* Van-DC12R90387; *V. parahemolyticus* 0394	Cheng et al. (2017)
			Positive	*St. aureus* B0125	
Bd-M1	Shrimp pond seawater and sediments	*V. parahaemolyticus* DX-1	Negative	*Ed. tarta* M1, M2, ET-1, ET-13, ET753; *V. alginolyticus* HY-1, Val; *V. harveyi* BK, Ocean-1; *V.* (*Lis.*) *anguillarum* E-3-11, M8-1; *V. parahaemolyticus* DX-1, DX-2, DX-3, DX-4, HY-2, Vp1, Vp2, 89001; *V. vulnificus* Vv-1, A1, A2	Chu et al. (2009)
BDH12, BDHSH06	Coastal seawater	*Sh. putrefaciens* strain 12, *V. parahaemolyticus* SH06	Negative	*Ae. salmonicida* 33; *Enterobacter salazakii* Bh07, Bh08; *Klebsiella oxytoca* 31; *Pantoea agglomerans* 30; *Ps. aeruginosa*; *Serratia ficaria* 15, 20; *Sh. putrefaciens* 12, 24, 27, 28, 34, 17,18, 35, 22, 29, 32; *V. alginolyticus* 1, 2, 3, 4, 10, 11, 13, 16, 19, 23, 1833; *V. cholerae* (non-01/0139) 6, 10-211, 11-114, 11-201, SWBC-A, SWBC-B; *V. fluvialis* Bh02, Bh03, Bh05, Bh11, Sh03, Sh0, Sh12, Sh13; *V. minicus* Bh10, Bh12,BH13, Bh15, Be08; *V. parahaemolyticus* 8, 9, 16, 15, 21, 25, 26, Vp plus, Vp minus, Sh06	Li et al. (2011)

(continued)

Table 1 (continued)

BALOs strains	Sources	Prey host	Gram nature	Bacteria that are susceptible to relevant BALOs	References
BDE-1	Coastal sediment	*Ba. subtilis* GIM1.136	Negative	*Klebsiella oxytoca* 31; *Ps. aeruginosa* 17, 22, 29, 32, 35; *Serratia ficaria* 20; *Sh. putrefaciens* 12, 27, 28, 34; *V. alginolyticus* 1, 4, 5, 10, 11, 16,19; *V. cholerae* (non-01/0139) 3, 14; *V. parahaemolyticus* 8, 9, 25	Li et al. (2018)
			Positive	*Enterococcus agglometans* 30	
BDW01, BDW02, BDW03, BDW04	Coastal sediment	*V. parahaemolyticus* (strain Vp minus)	Negative	*V. alginolyticus* 1, 2, 3, 4, 10, 11, 13, 16, 19; *V.* (*Lis.*) *anguillarum* Mvm; *V. cholerae* (non-01/0139) 6, SWBC-A, SWBC-B, 11-201, 11-114; *V. fluvialis* Bh02, Bh03, Bh05, Bh11, Sh03, Sh07, Sh12, Sh13; *V. hollisae* Be08; *V. minicus* Bh10, Bh12, Bh13, Bh15; *V. parahaemolyticus* 8, 9, 21, 25, 26, Sh06, Vp plus, Vp minus	Cai et al. (2008)
Bd. bacteriovorus Bd9301, Bd9302, Bd9305, Bd9306, Bd9308, Bd9311	Coastal seawater	*V.* (*Lis.*) *anguillarum* 89027	Negative	*Aeromonas* sp. 8903, 8946, 8930; *Es. coli; Plesiomonas* sp. 8910, 8917; *Ps. aeruginosa; V. alginolyticus* 8918, 8938; *V. cholerae*; *V. fluvialis* 8961, 8972, 8932; *V. harveyi* 8971; *V. (Lis.) anguillarum* 8927, 8962, 8988, LP9018, 8974, 8930; *V. metschnikovii*;	Yang and Huang (1997)

				V. parahaemolyticus; *Vibrio* sp. 8942,8943,8959, 8991	
			Positive	*Ba. subtilis*; *St. aureus*	
F16	Guts of sturgeon (*Ac. baerii*)	*Ae. hydrophila* S1 (sturgeon pathogen)	Negative	*Aeromonas* sp. ATCC7966, X1, W1-L, T3, R402L, RK1119, S1, 706C, 40142G, PK-T, XL2-T, LK-T, PL-R, S2-S	Cao et al. (2012)
Bd. bacteriovorus H16	Guts of sturgeon (*Ac. baerii*)	*Ae. hydrophila*	Negative	*Proteus* (*Pr.*) *mirabilis* strain ZL003, ZXS02, BYK64285, BYK64291; *Pr. vulgaris* strain TWN3; *Proteus* sp. strain ZL0057, BYK000419, BYK00098	Cao et al. (2014)
Bd. bacteriovorus H16	Guts of sturgeon (*Ac. baerii*)	*Ae. hydrophila*	Negative	*V. alginolyticus* BYK00019, BYK0834; *V. (Lis.) anguillarum* BYK0638; *V. cholerae* GYL, LD081008B-1; *V. harveyi* BYK00034, ZL0022; *V. parahaemolyticus* ZL0025, ZL0040; *V. vulnificus* BYK000965	Cao et al. (2015)

GIM denotes Guangdong Institute of Microbiology, Guangzhou, China

88.9% lysis rate) towards non-vibrio bacteria. Taking into account a similar finding that the BALOs in the Great Salt Lake preferentially prey upon bacteria isolated from the lake rather than bacterial isolates from ocean (Pineiro et al. 2004), and considering that partial 16S rDNA sequencing analysis showed BDH12 and BDHSH06 shared 99% sequence similarity (Li et al. 2011), we tend to believe that this preference could be the result of host adaptation. Once hosts are changed, they might well show different preferences after certain period of time. This is also supported by our own laboratory observations: when we change a BALOs' host, it initially needs 5–7 days or more for plaques to appear on the double-layer agar plates. After several rounds of subculturing, plaque formation usually takes much less time.

3.4 *Effect of BALOs on Fish or Shrimp Survivals in Challenge Tests*

To further confirm BALOs antibacterial activities and their potential applications in aquaculture, laboratory challenge tests are a step forward. Various laboratory challenge tests done so far have clearly proved that BALOs successfully protect tested fish or shrimp from pathogens attack, and improved their survival rates, with higher BALOs concentrations offering better protection efficiencies (Table 2).

Again, we took the work done by Kongrueng et al. (2017) as an example (Table 2). In the challenge test, it was divided into control and test groups, each with three subgroups. Control groups were subdivided into artificial sea water (ASW) only control, AHPND Vp-only control and *Bacteriovorax* sp. BV-A-only control, while test groups contained three different doses of BV-A groups, viz., 10^2, 10^4 and 10^6 PFU mL^{-1}. To start the test, shrimp AHPND pathogen Vp PSU5429 at a final concentration of 10^7 CFU (colony forming unit) mL^{-1}, was added to the AHPND Vp-only control and the three test groups that had already contained appropriate doses of BV-A. Fifteen minutes later, twenty whiteleg shrimp (*Lit. vannamei*) postlarvae (PL24) were added to each tank. The test was run for 7 days and shrimp mortalities were recorded daily. At the end of the 7-day test, over 90% of shrimp were dead in the AHPND Vp-only control, and 0% mortalities were recorded in ASW-only and BV-A-only controls. In the test groups, shrimp accumulative mortalities of 72.5, 62.5, and 47.5% were recorded in the subtest groups that contained BV-A at the final concentrations of 10^2, 10^4, and 10^6 PFU mL^{-1}, respectively. This result clearly demonstrated the protective effect of *Bacteriovorax* sp. BV-A on postlarval shrimp, with higher BV-A concentrations offering better protection efficiencies.

Most of the challenge tests done so far used the mode of bath challenge, viz., pathogens and BALOs as well as tested fish or shrimp were all added to the test tank waters, more or less simultaneously (Table 2). In this way, it gives BALOs time to act on the pathogens before the latter goes inside the fish/shrimp and causes

Table 2 Effect of BALOs on fish or shrimp survivals in challenge tests

BALOs strains	BALOs Final concentrations (PFU mL^{-1})	Ways of BALOs application	Test duration	Fish or shrimp tested	Species and doses in the challenge test	Fish or shrimp survival rates (%)	References
Bdellovibrio BD2082	0	BD2082 addition to waters and bath challenge simultaneously	6 days	Channel catfish (*Ictalurus punctatus*)	*Ae. hydrophila* S2027 at 10^7 CFU mL^{-1}	0	Zeng et al. (2004b)
	1×10^4					0	
	1×10^5					75	
	1×10^6					100	
	1×10^7					100	
	0	Pathogens dorsal muscle injection first, BD2082 addition to waters later			*Ae. hydrophila* S2027 at 10^7 CFU mL^{-1}	0	
	1×10^4					0	
	1×10^5					0	
	1×10^6					0	
	1×10^7					0	
BdC-1	0	BALOs addition and bath challenge simultaneously	14 days	Gibel carp (*Carassius auratus gibelio*)	*Ae. hydophila* J-1 at 5.0×10^8 CFU mL^{-1}	20	Chu and Zhu (2010)
	5×10^3					65	
	5×10^5					95	
Bd. bacteriovorus Bd-9-25922	0	BALOs addition and bath challenge simultaneously	11 days	Cyprinoid and grass carp (*Ctenopharyngodon idellus*)	*Ae. hydrophlia* at 10^8 CFU mL^{-1}	16.7	Yang et al. (2000)
	1×10^3					66.7	
	1×10^5					100	
Bd. bacteriovorus H16	0	BALOs addition and bath challenge simultaneously	7 days	Shrimp (*Penaeus vannamei*)	*V. cholerae* QH at 5×10^6 CFU mL^{-1}	0	Cao et al. (2015)
	5×10^3					47.7	
	1×10^4					63.3	
Bd. bacteriovorus H16	0	BALOs addition and bath challenge simultaneously	7 days	Shrimp (*Penaeus vannamei*)	*Pr. penneri* isolate NC at 5×10^6 CFU mL^{-1}	0	Cao et al. (2014)
	5×10^3					58.0	
	5×10^4					78.6	

(continued)

Table 2 (continued)

BALOs strains	BALOs Final concentrations (PFU mL^{-1})	Ways of BALOs application	Test duration	Fish or shrimp tested	Species and doses in the challenge test	Fish or shrimp survival rates (%)	References
[a]*Bdellovibrio* sp.	0	BALOs addition and bath challenge simultaneously	20 days	Crucian carp (*Ca. auratus*)	*Ae. hydrophila* at 10^5 CFU mL^{-1}	0	Huang et al. (2009)
	2mL[a]					70	
	4 mL[a]					100	
	8 mL[a]					100	
Bacteriovorax sp. BV-A	Control groups:	BV-A addition and bath challenge simultaneously	7 days	Postlarval shrimp (*Lit. vannamei*) (PL24)	AHPND Vp PSU5429 at 10^7 CFU mL^{-1}	100	Kongrueng et al. (2017)
	0 (ASW[b] only)					> 10	
	0 (AHPND Vp only)					100	
	1×10^6 (BV-A only)						
	Test groups:						
	1×10^2					27.5	
	1×10^4					37.5	
	1×10^6					52.5	

[a]BALOs concentration was not given
[b]2% artificial sea water

infections/diseases. Few were done by another way of challenge test, viz., muscle injection. Here, Zeng et al. (2004b) had carried out a challenge test by injecting pathogenic *Ae. hydrophila* S2027 into the dorsal muscle of channel catfish (*Ictalurus punctatus*), then instantly added BD2082 to the rearing waters (Table 2). They found that, compared to bathing challenge test that they had done simultaneously, all test fish died with no survival at all in the muscle injection challenge test at the end of the 6-day period. On the basis of this comparison, they concluded that BD2082 did not have curative effects and could be better used for prevention purposes. As pathogenic *Ae. hydrophila* S2027 and BD2082 are initially separated physically and bound to have a time lapse before the latter could predate the former, their conclusion looks not quite convincing scientifically. Nevertheless, it does indicate that BALOs should be at the infection/action sites earlier than the pathogens or potential pathogens, or at least at the same time or not too much later if they want to exert their protective roles.

This line of thinking was further supported by a study performed by Willis et al. (2016), who first injected into the hindbrain of zebrafish (*Danio rerio*) larvae with a lethal dose of *Shi. flexneri* M90T ($> 5 \times 0^3$ CFUs). Then, $1–2 \times 10^5$ PFUs of mCherry-*Bdellovibrio* was injected into the hindbrain ventricle of zebrafish larvae 30–90 min later. *Shigella* enumeration results demonstrated that zebrafish larvae injected with *Bdellovibrio* were able to control *Shigella* replication significantly better than those infected with *Shigella* alone. Moreover, *Bdellovibrio* could rescue zebrafish from lethal *Shigella* infection, increasing survival by ca. 35% at 72 h post injection.

3.5 *Effects of BALOs on Various Bacterial Numbers and Water Qualities*

Although most of the studies performed so far heavily relied on traditional culturing techniques to determine the effects of BALOs on the number of various bacteria, they did show that BALOs applications can indeed control the number of various bacteria, including total heterogenic bacteria counts, total vibrio counts, and/or some specific bacterial counts like *Edwardsiella* sp., at least for a certain period of time (Table 3). For an example, Wen et al. (2010) applied *Bacteriovorax* sp. strain DA5 (as identified with 16S rDNA sequencing by Wen et al. 2014) to the larviculture of white shrimp (*Lit. vannamei*) from nauplius stage ($N_{5–6}$) to mysis stage ($M_{1–2}$), and determined larval survival and metamorphosis rates, heterogenic bacterial and vibrio numbers (Table 4), as well as some water quality parameters (Table 3). At the end of the 9-day rearing test, they found that the high DA5 group significantly improved survival (20.83% vs. 10.42% in control and 9.09% in low DA5 group) and metamorphic rates (25% vs. 10% in control and 9.5% in low DA5 group) of mysis larvae (Table 5). When considering the reduction of bacteria by DA5, it was apparent that the amounts of heterotrophs and vibrios in rearing waters were reduced (a low DA5

Table 3 Effects of BALOs applications on various bacterial numbers and water quality

BALOs strains	BALOs final concentration (PFU mL^{-1})	Test duration/ ways of BALOs application	Reared organisms	Bacterial counts		Water quality parameters	References
				TCBC (%, or log CFU g^{-1}/mL^{-1})	TVC/TAC (%, or log CFU g^{-1}/ mL^{-1})		
Bd. bacteriovorus Bd2082	0	30 days / Bd2082 added to the test tanks filled with water from fish ponds	No fish	TCBC: decreased by 56.4[a]	Not given	Not given	(2004a)
	1.5×10^4			TCBC: decreased by 97.5[a]	Not given	Not given	
Bd. bacteriovorus	0	65 days / BALOs added to the test ponds	Grass carp (*Ctenopharyngodon idellus*)	TCBC: 6.62[b] grew to 6.77[b]	TAC: 6.38[b] grew to 6.58[b]	Compared with control, DO increased, NH_3-N, COD and sulfide contents decreased	Zhang et al. (2009a)
	50			TCBC: 6.63[b] down to 5.54[b]	TAC: 6.36[b] down to 5.43[b]		
	1×10^2			TCBC: 6.61[b] down to 6.49[b]	TAC: 6.41[b] down to 5.40[b]		
	1.5×10^2			TCBC: 6.65[b] down to 5.57[b]	TAC: 6.49[b] down to 5.41[b]		
Bd. bacteriovorus	0	7 days / BALOs added to the test ponds	Snakehead fish (*Ophiocephalus argus*)	Not given	TVC: increased by 0.21 ± 0.13[b]	Compared with control, NH_3-N and NO_2-N contents decreased ($p < 0.05$), DO increased ($p < 0.05$) and pH not changed	Li et al. (2008)
	75			Not given	TVC: decreased by 4.04 ± 0.62[b]		
	0				Not given	Not given	

Bdellovibrio sp. Bdm4		5 days / Bdm4 added to the test ponds	Crucian carp (*Ca. auratus*)	7.8 ± 0.07^{b} grew to 8.38 ± 0.07^{b} (*Edwardsiella* in gut)			Zhang et al. (2009c)
				$6.63 \pm 0{,}03^{b}$ grew to 7.03 ± 0.07^{b} (*Edwardsiella* on gill)			
				5.43 ± 0.08^{b} grew to 5.94 ± 0.16^{b} (*Edwardsiella* on skin)			
	1×10^{4}			7.36 ± 0.11^{b} down to 5.86 ± 0.06^{b} (*Edwardsiella* in gut)	Not given	Not given	
				6.44 ± 0.08^{b} down to 5.44 ± 0.14^{b} (*Edwardsiella* on gill)			

(continued)

Table 3 (continued)

BALOs strains	BALOs final concentration (PFU mL^{-1})	Test duration/ ways of BALOs application	Reared organisms	Bacterial counts		Water quality parameters	References
				TCBC (%, or log CFU g^{-1}/mL^{-1})	TVC/TAC (%, or log CFU g^{-1}/ mL^{-1})		
				5.27 ± 0.07 [b] down to 4.92 ± 0.05 [b] (*Edwardsiella* on skin)			
	0	3 days / Bdm4 in feed	Sea bream (*Sparus aurata*)	Not given	TVC: 0 (control was set as)	Not given	
	1×10^7			Not given	TVC: decreased by 87.7[a]	Not given	
BDH12 and BDHSH06	0	7 days / BDH12 and BDHSH06 added to the test ponds at 1:1 ratio	Oyster (*Ostrea rivularis*)	Not given	TVC: 8.0 grew to 9.0 (in waters).	Not given	Li et al. (2011)
					TVC: 5.82 to 10.0 (in intestine)		
	1×10^5			Not given	TVC: 8.09 ± 0.05 down to 2.39 ± 0.01[b] (in water)	Not given	
					TVpC: 8.02 ± 0.04 down to 2.33 ± 0.01[b] (in water)		
					TVC: 5.72 ± 0.02 down to 2.28 ± 0.01[b] (in intestine)		
					TVpC: 5.69±0.01 down to 2.24±0.04[b] (in intestine)		
BDW03	0	60 days / every 7 days, water was partially exchange with fresh seawater. BDW03 added to the test ponds again	Turbot (*Sc. maximus*)	TCBC: 3.9 ± 0.16[b] (in water)	TVC: 2.6 ± 0.23[b] (in water)	Initial data: pH 8.1 ± 0.097, NH_4-N 0.061 ±0.006 mg L^{-1}, NO_2-N 0.04 ± 0.008 mg L^{-1}, NO_3-N 2.03 ± 0.280 mg L^{-1}, DO 7.70 ± 0.280 mg L^{-1}	Guo et al. (2016)
				TCBC: 4.1 ± 0.09[c] (in intestine)	TVC: 3.2 ± 0.17[c] (in intestine)		
	1×10^5				TVC: 1.8 ± 0.27[b] (in water)		

				TCBC: 2.5 ± 0.13[b] (in water)		End data: pH 8.1 ± 0.120, NH_4-N0.058 ±0.002mg L^{-1}, NO_2-N 0.037±0.007 mg L^{-1}, NO_3-N 1.99 ± 0.530 mg L^{-1}, DO 7.65 ± 0.310 mg L^{-1}	
				TCBC: 3.0 ± 0.15[c] (in intestine)	TVC: 1.9 ± 0.10[c] (in intestine)		
BDH12	0	90 days / every 7 days, water was partially exchanged with fresh seawater. BDH12 added to the test ponds again	Abalone *(Ha. discus hannai)*	TCBC: 3.52 ± 0.03 grew to 6.14 ± 0.16[b] (in water)	TVC: 1.64 ± 0.14 grew to 3.22 ± 0.24[b] (in water)	Initial data: pH 8.2 ± 0.07, NH_4-N 0.02 ± 0.076 mg L^{-1}, NO_2-N 0.04 ± 0.002 mg L^{-1}, NO_3-N 2.16 ± 0.307 mg L^{-1}, DO 7.6 ± 0.31 mg L^{-1}	Guo et al. (2017)
				TCBC: 4.75 ± 0.03 grew to 7.09 ± 0.14[c] (in gut)	TVC: 3.84 ± 0.07 grew to 5.29 ± 0.12[c] (in gut)		
	1×10^5			TCBC: 3.50 ± 0.08 down to 2.07 ± 0.19[b] (in water)	TVC: 1.62 ± 0.13 down to 0.83 ± 0.09[b] (in water)	End data: pH 8.2 ± 0.12, NH_4-N 0.02 ± 0.94mg L^{-1}, NO_2-N 0.04 ± 0.001 mg L^{-1}, NO_3-N 2.15 ± 0.142 mg L^{-1}, DO 7.6 ± 0.31 mg L^{-1}	
				TCBC: 4.75 ±0.04 down to 2.98 ± 0.13[c] (in gut)	TVC: 3.82 ± 0.02 down to 1.75 ± 0.18[c] (in gut)		

(continued)

Table 3 (continued)

BALOs strains	BALOs final concentration (PFU mL^{-1})	Test duration/ ways of BALOs application	Reared organisms	Bacterial counts		Water quality parameters	References
				TCBC (%, or log CFU g^{-1}/mL^{-1})	TVC/TAC (%, or log CFU g^{-1}/ mL^{-1})		
BDH12	0	63 days / every 9 days entire pond of water was replaced with fresh seawater. BDH12 added to the test ponds again	Abalone (*Ha. diversicolor aquatilis*)	TCBC: 3.11^{c} grew to 7.22^{c} (in intestine)	TVC: 1.36^{c} grew to 5.42^{c} (in intestine)	Not given	Li and Cai (2014)
				TCBC: 3.05^{b} grew to 4.28^{b} (in water)	TVC: 1.25^{b} grew to 2.55^{b} (in water)		
	3.3×10^{5}			TCBC: 3.10^{c} grew to 5.96^{c} (in intestine)	TVC: 1.45^{c} grew to 3.39^{c} (in intestine)	Not given	
				TCBC: 3.16^{b} grew to 3.57^{b} (in water)	TVC: 1.16^{b} grew to 1.9^{b} (in water)		
DA5	0	9 days /DA5 added to the larval shrimp tanks in test groups	Larval shrimp (*Lit. vannamei*) (nauplius to mysis)	See Table 4 for the details	See Table 4 for the details	No significant difference on pH. COD and NH_3-N increased by 4.52 ± 0.22 / 0.65 ± 0.02 (Control), 4.54 ± 0.14 / 0.65 ± 0.03 (Low DA5), 5.02 ± 0.06 /0.77 ± 0.04 (High DA5), respectively.	Wen et al. (2010)
	1.15×10^{3}			See Table 4 for the details	See Table 4 for the details		
	1.15×10^{4}			See Table 4 for the details	See Table 4 for the details		

BDHSH06	0	85 days (every 7 days, water was partially exchanged with fresh seawater. BDHSH06 added to the test tanks)	Black tiger shrimp *(Penaeus monodon)*	TCBC:7.43 ± 0.12[b] (in water, BPERW/4HA)	TVC: 5.32 ± 0.07[b] (in water, BPERW/4HA)	Not given	Li et al. (2014)
				TCBC: 10.52 ± 0.25[c] (in intestine, BPERW/4HA)	TVC: 6.51 ± 0.04[b] (in intestine, BPERW 4HA)		
	1×10^5			TCBC: 5.20 ± 0.09[b] (in water, BPERW/4HA)	TVC: 3.55 ± 0.13[b] (in water, BPERW/4HA)	Not given	
				TCBC: 6.04 ± 0.13[c] (in intestine, BPERW/4HA)	TVC: 5.18 ± 0.19[c] (in intestine, BPERW/4HA)		

BPERW/4HA denotes before the partial exchange of rearing water/4 h after BDHSH06 addition; TCBC denotes total cultivable bacterial counts; TVC denotes total vibrio counts, TVpC denotes total *V. parahaemolyticus* counts, TAC denotes total aeromonad counts: [a]%.; [b]log CFU mL^{-1}, [c]log CFU g^{-1}

Table 4 Effect of *Bacteriovorax* sp. DA5 on the heterogenic bacteria and vibrio numbers in rearing waters of white shrimp (*Lit. vannamei*) (adapted and modified from Wen et al. 2010)

	Heterotrophic bacteria ($\times 10^5$ CFU mL^{-1})			Vibrios ($\times 10^3$ CFU mL^{-1})		
Test days∗ (Larval stage)	Control	Low DA5	High DA5	Control	Low DA5	High DA5
0 (N_5-N_6)	6.67 ± 1.74^a	4.90 ± 1.41^a	6.48 ± 1.31^a	13.60 ± 0.57^a	11.38 ± 3.64^a	14.47 ± 1.08^a
0.5 (N_6-Z_1)	9.41 ± 1.90^a	9.00 ± 0.38^a	6.38 ± 0.26^a	18.23 ± 1.38^a	20.13 ± 5.69^a	13.38 ± 0.25^a
1 (N_6-Z_1)	174.33 ± 1.41^a	174.00 ± 8.49^a	144.50 ± 6.84^b	94.25 ± 10.96^a	109.50 ± 2.83^a	92.75 ± 9.55^a
2 (Z_1-Z_2)	22.33 ± 0.94^a	16.00 ± 0.47^a	11.67 ± 3.77^a	15.50 ± 2.83^a	16.00 ± 1.41^a	13.25 ± 1.77^a
3 (Z_1-Z_2-Z_3)	11.55 ± 2.57^a	9.60 ± 1.23^a	2.92 ± 0.87^b	5.35 ± 0.14^a	4.88 ± 0.25^a	3.35 ± 0.57^b
5 (Z_2-Z_3)	3.28 ± 0.49^b	4.58 ± 0.97^b	7.92 ± 1.06^a	0.83 ± 0.09^a	0.85 ± 0.21^a	2.02 ± 0.64^a
7 (Z_3-M_1)	32.00 ± 15.56^a	25.25 ± 1.06^a	15.50 ± 7.07^a	6.80∗∗	8.15∗∗	3.05 ± 1.27
Total increment (%)	864.71	651.71	336.56			
Total reduction (%)				52.01	45.74	72.22–88.55

Different superscript letters (^{a}, ^{b}) in the same line of data showed significant difference ($P < 0.05$) (Wen et al. 2010); ∗Test Day 0 meant samplings were done 30 min before adding DA5; ∗∗represented only one in two replicate samples could be counted effectively; No data were available on Test day 9 because of inappropriate dilutions on 2216E and TCBS plates

Table 5 BALOs applications in aquaculture practices and their effects on growth and survival of reared organisms

BALOs strains	BALOs final concentrations (PFU mL^{-1})	Test duration (BALOs added to the ponds directly)	Reared organisms	Survival rates (%)	Length gain (%)[a]	Weight gain (%)[b]	References
BDH12	0	90 days (every 7 days, water was partially exchanged with fresh seawater. BDH12 added to the test ponds)	Abalone juvenile (*Ha. discus hannai*)	41.8 ± 3.36	216 ± 17	4168 ± 47	Guo et al. (2017)
	1×10^5			63.3 ± 1.87	272 ± 15	6834 ± 39	
BDW03	0	60 days (every 7 days, water was partially exchange with fresh seawater. BDW03 added to the test ponds)	Turbot (*Sc. maximus*)	81 ± 3.2	56.7 ± 2.1	248.2 ± 5.3	Guo et al. (2016)
	1×10^5			92 ± 2.8	78.6 ± 1.5	387.1 ± 4.6	
BDHSH06	0	85 days (every 7 days, water was partially exchanged with fresh seawater. BDHSH06 added to the test tanks)	Black tiger shrimp (*Penaeus monodon*)	31.0 ± 2.1	86.0 ± 11.1	4.21 ± 1.56	Li et al. (2014)
	1×10^5			48.1 ± 1.2	99.8 ± 10.0	6.36 ± 1.50	
BDH12	0	63 days (every 9 days, entire pond of water was exchanged with fresh seawater. BDH12 added to the test ponds)	Abalone (*Ha. diversicolor aquatilis*)	27 ± 2.8	13.49 ± 0.1	47.33 ± 4.25	Li and Cai (2014)
	3.3×10^5			57 ± 6.8	15.43 ± 0.1	55.21 ± 4.59	
BDFM05	0	42 days (every 7 days, entire pond of water was exchanged with fresh seawater. BDFM05 added to the test ponds)	Abalone spat (*Ha. discus hannai*)	45.8	0 (average shell length: 4.332 mm)	Not given	Xiao and Cai (2011)
	1×10^3			75.8	31.7 (average shell length: 5.707 mm)	Not given	
	1×10^4			80.9	46.4 (average shell length: 6.343 mm)	Not given	

(continued)

Table 5 (continued)

BALOs strains	BALOs final concentrations (PFU mL^{-1})	Test duration (BALOs added to the ponds directly)	Reared organisms	Survival rates (%)	Length gain (%)[a]	Weight gain (%)[b]	References
DA5	0	9 days (DA5 added to the test groups)	Larval shrimp (*Lit. vannamei*) (from nauplius to mysis stage)	10.42 (metamorphosis rate: ca. 10%)	Not given	Not given	Wen et al. (2010)
	1.15×10^3			9.09 (metamorphosis rate: ca. 9.5%)	Not given	Not given	
	1.15×10^4			20.83 (metamorphosis rate: ca. 25%)	Not given	Not given	

[a]A percentage of the length gain (%) was performed by the shell length difference between the test group and control divided by the shell length of control. Set the shell length gain (%) in control as zero

[b]A percentage of the weight gain (%) was performed by the body weight difference between the test group and control divided by body weight of control. Set the weight gain (%) in control as zero

concentration of 1.15×10^3 PFU mL^{-1}) or significantly ($p < 0.05$) reduced (a high DA5 concentration of 1.15×10^5 PFU mL^{-1}) in the first 3 days of the test (Table 4); that is, the heterogenic bacterial numbers, based on 2216E agar plate counts, increased from $6.67 \pm 1.74 \times 10^5$ CFU mL^{-1} and $4.90 \pm 1.41 \times 10^5$ CFU mL^{-1} on Day 0 to $11.55 \pm 2.57 \times 10^5$ CFU mL^{-1} and $9.60 \pm 1.23 \times 10^5$ CFU mL^{-1} on Day 3 in the control and low DA5 groups, respectively, while their number was reduced from $6.48 \pm 1.31 \times 10^5$ CFU mL^{-1} to $2.92 \pm 0.87 \times 10^5$ CFU mL^{-1} in high DA5 group during the same period of time (Table 4). Heterogenic bacterial numbers then gradually rose in the high DA5 group, or went further down on day 5 and then rose again on Day 7 in the control and low DA5 groups (no data was available on Day 9 due to an over dilution of that days samples, as the authors explained). Overall, the increments of heterogenic bacteria in the control, low DA5 and high DA5 groups over the 7-day test period were 864.71%, 651.71% and 336.56%, respectively (Table 4). These data clearly indicated that DA5 was effective in the control of heterogenic bacteria numbers in postlarval rearing tanks, with higher efficiencies at relatively higher concentrations.

A similar trend was also noted in the total vibrio counts (Table 4), with reductions over the 7-day period in the control, low DA5 and high DA5 groups at 52.01%, 45.74% and 72.22–88.55%, correspondingly. Once more, these data fully demonstrate the effectiveness of *Bacteriovorax* sp. strain DA5 in the control of vibrios in postlarval rearing tanks.

With respect to water quality, there were no significant differences throughout the test period in pH, COD, and ammonia-N (NH_3-N) contents in waters among control, low DA5 and high DA5 groups, with the exception that the NH_3-N content in high DA5 group at mysis I-II stage (M_{1-2}, near the end of the test) increased significantly (Table 3). This difference could be due to the higher amount of feed given to high DA5 group as it had more postlarvae, rather than the effects directly exerted by BALOs (Wen et al. 2010).

On further reviewing existing documentation discussing the effects of BALOs on water quality, only two pieces of work showed the improvements after BALOs applications. The first one was done by Li et al. (2008), who showed that after a 7-day application of *Bd. bacteriovorus* at a dose of 0.75 mL per square meter of 1.0×10^8 PFU mL^{-1} stock, the NH_3-N, NO_2-N contents were significantly decreased ($p < 0.05$), and DO values were significantly increased ($p < 0.05$), but pH was not significantly changed ($p > 0.05$) (Table 3). The second one was done by Zhang et al. (2009a), who also demonstrated the increase of DO, and the decrease of NH_3-N and sulfide contents (Table 3). These two studies both pointed to the improvement of water quality by BALOs in aquaculture, although to various extents. On the other hand, Gou et al. (2016, 2017) also examined the effects of BALOs on water quality and showed no significant differences (Table 4).

As PCR-DGGE is a relatively powerful tool to provide information into a microbial community structure qualitatively and quantitatively, Chen et al. (2019) employed it to study the effects of *Bacteriovorax* sp. N1 on the bacterial community structures in aquaculture of both seawater sea cucumber (*Ap. japonicus*) and freshwater red carp. Bacterial community structures from the rearing waters were

analyzed using PCR-DGGE analysis over the 48 h-test period. They showed that in freshwater red carp rearing waters, the dominant vibrio and δ-*Proteobacteria* decreased significantly after 12 h of *Bacteriovorax* sp. N1 application, but *Ps. fluorescens* and *Thalassobius aestuarii* increased. In seawater *Ap. japonicus* rearing waters, the dominant δ-*proteobacteria* bacterium became a non-dominant one at 12 h while *Albirhodobacter* became the new dominant bacterium. Based on these results, the authors concluded that *Bacteriovorax* sp. N1 could not only lyse vibrios, δ-*proteobacteria* and many other Gram-negative bacteria, but also increase the number of some other bacteria in both seawater and freshwater aquaculture environments. Nevertheless, they also noted that *Bacteriovorax* sp. N1 concentrations decreased to its lowest level within 24 h and, therefore, it should be replenished per 24 h if it were used to control vibrios continuously.

The decrease of *Bacteriovorax* sp. N1 concentrations with time could well explain a phenomenon we noted in the study by Wen et al. (2010), that bacterial numbers, both heterotrophs and vibrios, went down first in the midst of the test period, and then rose up near the end of the test. The rise of both heterotrophs and vibrio numbers may well mean the decrease of DA5 numbers in the rearing waters. Unfortunately, the authors did not enumerate BALOs/DA5 numbers during the test period. This makes this association remain theoretical.

3.6 BALOs Applications in Aquaculture Practices

Various BALOs application studies have been performed in shrimp, turbot and abalone aquaculture practices with a view to control the overgrowth of various bacteria (including pathogens or potential pathogens) (Tables 3 and 4) and to enhance the growth and survival of reared organisms (Table 5).

In larviculture, Wen et al. (2010) applied *Bacteriovorax* sp. strain DA5 to white shrimp (*Lit. vannamei*), from nauplius stage ($N_{5–6}$) to mysis stage ($M_{1–2}$). They found that at the end of the 9-day test, shrimp survival and metamorphic rates were much higher in high DA5 group (20.83% and 25%, respectively) than those in control and low DA5 group (10.42%, 9.09% and 10%, 9.5%, correspondingly) (Table 5). A similar finding was also demonstrated by Xiao and Cai (2011) in abalone larviculture. They revealed that in comparison to controls with a 45.8% survival rate, BALOs BDFM05 application led to higher rates of survival (65.50% and 76.64% higher) in low and high BDFM05 groups, respectively (Table 5). Their shell length gain was 31.74% and 46.42% higher as compared to control (Table 5).

In grown out aquaculture, Li et al. (2014), Li and Cai (2014), and Guo et al. (2016, 2017) all demonstrated that BALOs applications brought about higher growth and survival rates of reared organisms as compared to controls (Table 5). That is, Li et al. (2014) performed an 85-day rearing test on black tiger shrimp (*Penaeus monodon*) and showed that the survival rate, body length and weight gains of black tiger shrimp were 70.59%, 46.60% and 196.60% higher respectively, in BDHSH06 group compared to control. On abalone tests, Gou et al. (2017)

performed a 90-day rearing test on abalone (*Ha. discus hannai*) and showed that the survival rate, body length and weight gains of abalone were 69.54%, 44.22% and 66.78% higher respectively, in BDH12 group as compared to control, while Li and Cai (2014) ran a 63-day rearing test on abalone (*Ha. diversicolor aquatilis*) and showed that the survival rate, body length and weight gains of abalone were 163.64%, 15.98% and 38.81% higher in BDH12 group compared to control, correspondingly. Regarding fish tests, Gou et al. (2016) performed a 60-day test on turbot (*Sc. maximus*) and showed that the survival rate, body length and weight gains of abalone were 21.85%, 46.70% and 61.26% higher in BDW03 group as compared to control, respectively.

To explore possible links among bacterial numbers with survival and growth rates of those reared organisms, we have performed statistical analyses (Tables 6 and 7). Statistical analyses were carried out using IBM SPSS Statistics (V23, New York, USA). Correlations among various parameters, including various bacterial numbers, survival rates, shell (body) length and body weight gains, as well as added BALOs concentrations, were assessed using Pearson's correlation coefficient, r. In terms of the strength of relationships, the value of the correlation coefficient varies between +1 and − 1. The meanings are as follows:

(i) A correlation coefficient of 1 means that for every positive increase in one variable, there is a positive increase of a fixed proportion in the other.
(ii) A correlation coefficient of −1 means that for every positive increase in one variable, there is a negative decrease of a fixed proportion in the other.
(iii) Zero means that for every increase, there isn't a positive or negative increase. The two just aren't related.

We first analyzed those relevant end-of-a-test data (viz., data at the end point of a test, instead of a series of data covering the beginning and the end as done in some original references) as shown in Table 5 and gave out the statistical results in Table 6.

Although analyses on the end-point data may not be as robust as we would like due to the limitation of available published data in the references, they at least show the trends of developments.

Pearson analysis on TCBC (total culturable bacterial counts), TVC (total vibrio counts), survival/metamorphosis rates, body length and weight gains revealed that in shrimp larviculture (Wen et al. 2010), TCBC had no significant correlations with the rates of larval survival ($r = -0.901$) or metamorphosis ($r = -0.927$). While TVC had a significant negative correlation with survival rates ($r = -0.997$), it had no significant negative link with metamorphosis rates ($r = -0.991$). Unfortunately, we were not able to perform such analyses on the study done by Xiao and Cai (2011) as they did not present data on TCBC and/or TVC. In the grown out aquaculture (Li et al. 2014; Li and Cai 2014; Guo et al. 2016, 2017), it is quite clear that the end-point data of the tests, viz., TCBC and TCVC, both in waters and intestines, all have very strong negative impacts ($r = -1.000$) on the survivals, length gains and weight gains of the reared organisms (Table 6).

Table 6 Pearson's correlations between relevant bacterial numbers and survival or length (gain) or weight (gain) of reared organisms[a]

BALOs strains	Sampling sites	Treatment	TCBC log CFU g^{-1}or mL^{-1}	TVC log CFU g^{-1} or mL^{-1}	S%	L%	W%	M%	Correlations								References
									TCBC × S	TCBC × L	TCBC × W	TVC × S	TVC × L	TVC × W	TCBC × M	TVC × M	
BDW03	Intestine	Control	4.1	3.2	81	56.7	248.2		**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)			Guo et al. (2016)
		Test	3.0	1.9	92	78.6	387.1										
	Water	Control	3.9	2.6	81	56.7	248.2		**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)			
		Test	2.5	1.8	92	78.6	387.1										
BDH12	Gut	Control	7.09	5.29	41.8	216	4168		**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)			Guo et al. (2017)
		Test	2.98	1.75	63.3	272	6834										
	Water	Control	6.14	3.22	41.8	216	4168		**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)			
		Test	2.07	0.83	63.3	272	6834										
BDH12	Intestine	Control	7.22	5.42	57	13.49	47.33		**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)			Li and Cai (2014)
		Test	5.96	3.39	27	15.43	55.21										
	Water	Control	4.28	2.55	57	13.49	47.33		**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)			
		Test	3.57	1.9	27	15.43	55.21										
BDHSH06	Intestine	Control	10.52	6.51	31.0	86.0	4.21		**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)			Li et al. (2014)
		Test	6.04	5.18	48.1	99.8	6.36										
	Water	Control	7.43	5.32	31.0	86.0	4.21		**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)	**(r = −1.0)			
		Test	5.20	3.55	48.1	99.8	6.36										
DA5	Water	Control	6.51	3.83	10.42			10	NS (r = −0.901)	/	/	*(r = −0.997)	/	/	NS (r = −0.927)	NS (r = −0.991)	Wen et al. (2010)
		Low DA5	6.4	3.91	9.09			9.5									
		High DA5	6.19	3.48	20.83			20.5									

[a]Due to the limitations of available data in most of the references, most of the Pearson analyses done here were on the data from the end points of the tests, instead of a series of data covering the whole test period. The *TCBC/TVC* units used for intestine samples were log CFU g^{-1}; The *TCBC/TVC* units used for water samples were log CFU mL^{-1}; *S* denotes survival rates, *L* denotes body (or shell) length or length gains, *W* denotes body weight or weight gains, *M* denotes metamorphosis rates; *denotes significant correlation ($p < 0.05$); ** denotes extremely significant correlation ($p < 0.01$); *NS* not significant.

Table 7 Pearson's correlations between BALOs additions and relevant bacterial numbers, survival or (shell) length (gain) or body weight (gain) of reared organisms[a]

BALOs strains	BALOs added concentration PFU mL^{-1}	Sampling sites	TCBC log CFU g^{-1} or mL^{-1}	TVC log CFU g^{-1} or mL^{-1}	S%	L%	W%	M%	Correlations						References
									BALOs × TCBC	BALOs × TVC	BALOs × S	BALOs × L	BALOs ×W	BALOs × M	
BDW03	Control: 0	Water	3.9	2.6	81	56.87	248.2		**(r = −1.0)	**(r = −1.0)	**(r = 1.0)	**(r = 1.0)	**(r = 1.0)		Guo et al. (2016)
	Test: 1×10^5		2.5	1.9	92	78.6	387.1								
	Control: 0	Intestine	4.1	3.2	81	56.87	248.2		**(r = −1.0)	**(r = −1.0)	**(r = 1.0)	**(r = 1.0)	**(r = 1.0)		
	Test: 1×10^5		3.0	1.8	92	78.6	387.1								
BDH12	Control: 0	Water	6.14	3.22	41.8	216	4168		**(r = −1.0)	**(r = −1.0)	**(r = 1.0)	**(r = 1.0)	**(r = 1.0)		Guo et al. (2017)
	Test: 1×10^5		2.07	0.83	63.3	272	6834								
	Control: 0	Gut	7.09	5.29	41.8	216	4168		**(r = −1.0)	**(r = −1.0)	**(r = 1.0)	**(r = 1.0)	**(r = 1.0)		
	Test: 1×10^5		2.98	1.75	63.3	272	6834								
BDH12	Control: 0	Water	4.28	2.55	27	13.49	47.33		**(r = −1.0)	**(r = −1.0)	**(r = 1.0)	**(r = 1.0)	**(r = 1.0)		Li and Cai (2014)
	Test: 3.3×10^5		3.57	1.9	57	15.43	55.21								
	Control: 0	Intestine	7.22	5.42	27	13.49	47.33		**(r = −1.0)	**(r = −1.0)	**(r = 1.0)	**(r = 1.0)	**(r = 1.0)		
	Test: 3.3×10^5		5.96	3.39	57	15.43	55.21								
BDHSH06	Control: 0	Water	7.43	5.32	31.0	86.0	4.21		**(r = −1.0)	**(r = −1.0)	**(r = 1.0)	**(r = 1.0)	**(r = 1.0)		Li et al. (2014)
	Test: 1×10^5		5.20	3.55	48.1	99.8	6.36								
	Control: 0	Intestine	10.52	6.51	31.0	86.0	4.21		**(r = −1.0)	**(r = −1.0)	**(r = 1.0)	**(r = 1.0)	**(r = 1.0)		
	Test: 1×10^5		6.04	5.18	48.1	99.8	6.36								
DA5	Control: 0	Water	6.51	3.83	10.42			10	NS (r= −0.968)	NS (r=−0.965)	NS (r = 0.981)			NS (r = 0.991)	Wen et al. (2010)
	Low DA5: 1.15×10^3		6.4	3.91	9.09			9.5							
	High DA5:														
	1.15×10^4		6.19	3.48	20.83			20							
								5							
BDFM05	Control: 0				45.8	0					NS (r = 0.681)	NS (r = 0.801)			Xiao and Cai (2011)
	Test: 1×10^3				75.8	31.7									
	Test: 1×10^4				80.9	46.4									

[a]Due to the limitations of available data in most of the references, most of the Pearson analyses done here were on the data from the end points of the tests, instead of a series of data covering the whole test period. The *TCBC/TVC* units used for intestine samples were log CFU g^{-1}; The *TCBC/TVC* units used for water samples were log CFU mL^{-1}; *S* denotes survival rates, *L* denotes length or length gains, *W* denotes weight or weight gains, *M* denotes metamorphosis rates; ∗denotes significant correlation ($p < 0.05$); ∗∗denotes extremely significant correlation ($p < 0.01$); *NS* not significant.

We then went on to analyze effects of BALOs additions on the test-end-point TCBC and TCVC, both in waters and intestines, and survivals, as well as body (shell) length gains and weight gains of the reared organisms (Table 7).

It is surprising to note that in both shrimp (Wen et al. 2010) and abalone (Xiao and Cai 2011) larviculture, BALOs added concentrations display no significant correlations with TCBC, TVC, survival or metamorphosis rates (Table 7). In abalone and turbot grow-out aquaculture, BALOs added concentrations did have significant negative links with the test-end-point TCBC and TVC ($r = -1.000$), in waters or guts, and positive correlations with survival, body (shell) length gains and weight gains ($r = 1.000$).The finding that showed no statistically significant links between BALOs added concentrations and the test-end-point TCBC, TVC, survival or metamorphosis rates indicate the complexities of larviculture, and more work need to be done before their potential interrelationships could be established.

Strong positive correlations between BALOs added concentrations and growth parameters (survival, body length and weight gains) were supported by the studies of Yang et al. (2016) and Xiong et al. (2017) who revealed a beneficial link between BALOs abundance in guts and shrimp health or growth. This is also supported by Iebba et al. (2013), who revealed a higher prevalence and abundance of *Bdellovibrio bacteriovorus* in the human gut of healthy subjects, implying that BALOs do contribute to the health, and by Shatzkes et al. (2017), who evaluated the effect of predatory bacteria on the gut bacterial microbiota in rats and predicted the changes in bacterial populations due to exposure to *Bd. bacteriovorus* would contribute to health.

4 BALOs Applications in the Infection Treatments in Aquaculture

Much rare work has been done, so far, regarding the use of BALOs to treat infections of reared organisms in aquaculture practice. Only Chen and Cai (2011) had conducted such a study.

Recognizing that hemorrhagic symptoms in the mouths of farmed turbot (*Sc. maximus*) was caused by *V. splendidus* (Angulo et al. 1994), Chen and Cai (2011) collected juvenile turbot (55 ± 2.5 g body weight) with some signs of red mouth symptom. They divided these fish into several groups, including groups of control, low BDM01 (10^3 PFU mL^{-1}), medium BDM01 (10^5 PFU mL^{-1}) and high BDM01 (10^7 PFU mL^{-1}). During the test, appropriate amounts of BDM01 were added every 2–3 days to the rearing waters to bath fish and to maintain BDM01 concentrations. No water flow was allowed during the test period so as to avoid BDM01 being diluted and the possible coming-in of new pathogens. Tests were run for 7 days. In comparison with a 47% survival rate in the control, the three different test groups achieved 98.67%, 99.33%, and 100% survival rates. Red mouth signs became fainter or disappeared in most of the fish in the test groups.

Though the use of BDM01 to treat red mouth symptoms in juvenile turbot proved to be successful, it does not mean it will be feasible in other occasions. There are four reasons to this. Firstly, the red mouth infections were at their very early stages as most fish with very faint reddish lips were selected. Secondly, the rearing temperature was relatively appropriate for the BDM01 to act (21–22 °C). Thirdly, the traditional flow-through water exchange was stopped. This should avoid the coming-in of any potential new pathogens and help maintain BDM01 concentrations. Fourthly, BDM01 was a relatively powerful lytic strain with higher efficiencies (unpublished data). This made it work faster in the elimination of vibrios.

5 Conclusions

Through the above comprehensive review on the relevant high quality documented studies, we can conclude that BALOs are naturally ubiquitous in aquaculture environments and even in the guts of reared organisms. They do show strong antibacterial activities against various Gram-negative bacteria and even some Gram-positives, including pathogens or potential pathogens in aquaculture. It is also quite clear that BALOs definitely have a role to play in aquaculture, in terms of controlling the number of bacteria, be it pathogenic or potentially pathogenic, and promoting growth and survival of the cultured organisms. Whether or not BALOs could improve water qualities, directly or indirectly, requires more rigorous work to be performed before definite answers could be given.

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