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Interactions of microorganisms and their use as biocontrol agents in aquaculture

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Abstract : The function and activity of microorganisms are the key factors in understanding the quality of the aquacultural environment. In fact, both harmful and useful microbes exist in aquaculture water that directly affect fish growth. Among these microorganisms, useful bacteria that can repress pathogenic microbes in the process of microbial antagonism and are utilized in biological production are called biocontrol (biological control) agents (BCAs). BCAs were applied to aquaculture production in this study to prevent bacterial and viral diseases in fishes and crustaceans. This paper describes the results of a study of biocontrol for fish and crustacean production, and also reviews research reports on the use of microorganisms as BCAs in aquacultural processes.

Keywords : *biocontrol, probiotics, antagonism, microorganisms, virus, aquaculture, fish, crustaceans*

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The main purpose of this study was to find and utilize microorganisms that promote fish growth, while at the same time repressing the growth of pathogenic microorganisms. The results are presented here, in addition to a review of the use of microorganisms as biological control (biocontrol) agents (BCAs) in aquaculture.

Antagonism among microbes is a naturally occurring phenomenon, through which pathogens can be killed or reduced in numbers. In order to apply such biocontrol to the aquacultural environment, BCAs that can repress the growth of pathogenic bacteria and viruses were sought. Initially, microorganisms that promote fish and crustacean growth were isolated, since BCAs should not be harmful to them. Prawns (*Penaeus monodon*) were cultured with and without soil extracts (the source of organic matter). Higher survival and molt rates of prawn larvae were obtained in the experiment that contained soil extracts, the bacterial strain that promoted the growth of the prawn larvae being isolated (MAEDA and LIAO, 1992). The same bacterial strains also promoted the growth of a crab (*Portunus trituberculatus*) (MAEDA *et al.*, 1992; MAEDA, 1999). Following this procedure, several other bacterial strains that promoted the growth of fishes, such as striped jack, sea bream and

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flatfish were also isolated (MAEDA, 1999).

These useful bacteria were marked with a fluorescent dye following the method of SHERR *et al.* (1987), and fed live to rotifers (*Brachionus plicatilis*) and crab larvae (*Portunus trituberculatus*). Under an epifluorescent microscope, the stained bacteria could be seen inside the guts of the rotifers and crab larvae. Thus, the utility of bacteria as live food can be determined from the survival data of their predators, as well as through direct observation of the former following ingestion (MAEDA and LIAO, 1994; MAEDA, 1999).

Vibrio anguillarum and infectious hematopoietic necrosis virus (IHNV) were used as microbial pathogens to test whether or not isolated microbial strains could repress the growth of those pathogens. Accordingly, several bacterial strains that strongly repressed the growth of the pathogenic microorganisms were obtained. Determination of anti-viral activity in isolated bacteria indicated that bacteria that showed vibrio-static activity were also able to repress the infectious activity of the virus (MAEDA *et al.*, 1997).

One of these BCAs was applied to the culture of crab (*Portunus trituberculatus*) larvae that were found to be infected by a pathogenic *Vibrio* sp. Before this application, crab culture methodology included the addition of several antibiotics to the larval rearing water, such treatment being initially able to repress the growth of pathogenic *Vibrio* sp., however, the appearance of resistant microbes (mainly fungi) killed all of the larvae within a few days. Infection of larvae by pathogens interfered significantly larval production wherein whole batches of diseased larvae were abandoned and a new production cycle initiated. Although the shortcomings of antibiotic use were apparent, few if any alternative means for controlling disease were known. It was therefore essential that new approaches should be adopted, wherein the antagonism of certain microorganisms could be used to repress other pathogenic microbes in aquaculture systems.

Subsequently, the addition of a bacterial strain as a biocontrol agent, instead of antibiotics, to the *Portunus trituberculatus* larval culture facility was found to improve growth and protect larvae from pathogens. Among the bacterial assemblages monitored, the added bacterial strain dominated the bacterial populations, *Vibrio* spp. counts decreasing or becoming undetectable in seawater. In this way, production of crab larvae was greatly increased (MAEDA and NOGAMI, 1989; NOGAMI and MAEDA, 1992; MAEDA, 1999). Two possible explanations for the reduction in concentrations of *Vibrio* spp. when the BCA was added are: (1) the production (although not high) of vibrio-static reagents by the BCA, and (2) niche exclusion between the zymogenous bacteria and BCA. The latter is particularly important in controlling microbial communities. In experiments not involving the addition of a BCA, survival rates of larvae from zoea I to zoea IV were high, larvae not always being infected with pathogenic microbes. However, the larvae died on reaching the megalopa I growth stage in many experiments, probably because of nutrient deficiency. These data suggest that the use of the BCA might improve the physiological state of the larvae by serving as a nutrient source for growth (NOGAMI and MAEDA, 1992).

One of the bacterial strains used in aquacultural processes showed an ability to prevent infection of fish larvae by Striped Jack Nervous Necrosis Virus, baculo-like viruses and irido virus. When this strain was added to water containing the larvae of the *Penaeus* prawns, striped jack and sea bream, the survival rates of these larvae were much higher than those without the bacterial strain, all fish larvae dying due to viral disease in the latter experiments (MAEDA *et al.*, 1997; MAEDA, 1999). Viruses spread from infected fish to healthy fish through the seawater, thereby reducing the fish numbers gradually or rapidly. However, BCAs could help to inhibit the spread of viruses among fishes. In addition, if fish fed on such BCAs, a probiotic effect might be strengthening of the immune system. With such useful effects and features, BCAs could prove to be effective in protecting fish from the spread of bacterial and viral diseases in aquaculture.

Following the feeding of an artificial compound feed (ACF)/BCA mixture to fish, the bacteria contained in residual ACF and feces

after digestion sank to the sediment. Eventually, bacteria, including BCAs, degraded the organic matter in the sediment (MAEDA, 1999). In the sediment, the many benthic animals that feed on detritus, microorganisms and other small animals, move and agitate particles, thereby allowing greater penetration of oxygen-rich water into the sediment (bioturbation). In heavily stagnant and eutrophicated sediments, if such BCAs are added and grow well, the bacteria stimulate the growth of benthic animals, resulting in accelerated bioturbation and material processing, which in turn stimulates the growth of various other microorganisms and animals. In this manner, the activity levels of these organisms at various trophic levels is accelerated and an improved sedimentary environment evolves.

Through these studies, the author determined that a bacterium exists that promotes the growth of microalgae, rotifers and fishes at the same time. Since microalgae – rotifers – fish are linked in the food chain, this bacterium functions well as a growth promoter on each component, and this microbial process is termed the "microbial line" in aquaculture.

Following is a review of the interactions of microorganisms and their use as biocontrol agents in aquaculture.

2. Biocontrol Agents (BCAs) and Probiotics

Biological control (biocontrol) utilizes the naturally occurring antagonism between organisms, having been frequently used to enhance the activity of natural antagonists to repress growth or kill pathogenic organisms in agriculture (LANDIS *et al.*, 2000). The method is especially familiar in the example of the bacterium, *Bacillus thuringiensis,* which infects the mouth of pathogenic insects and eventually kills them (KERR, 1972 and 1980), the process having been commercialized in Europe and North America where several thousands of tons of the former are used in agriculture. The results have encouraged further studies on the use of viruses, fungi and protozoa as biocontrol agents to eliminate pathogenic organisms. The three main biocontrol methods include classical (augmentative), conservation and integrated biocontrol. In the classical method, exotic agents are used against pathogens being released or those released augmentatively (GURR and WRATTEN, 1999). The use of conservation biocontrol in conjunction with classical or augmentative methods, is called integrated biocontrol. As a method of habitat manipulation, conservation and integrated biocontrol include actions with multiple functions, such as sowing one plant near another in order to release materials, whilst having a negative influence on the plant's natural enemies. Thorough research of the ecosystem and careful management is required to manage these trade-offs.

LILLY and STILLWELL (1965) coined the term "probiotics" to describe substances produced by one protozoan which stimulated another. Subsequently the term became used to describe animal feed supplements that had a beneficial effect on the host animal by affecting its gut flora (PARKER, 1974). FULLER (1989) used "probiotic" to describe live microbial feed supplements that beneficially affected the host animal by improving its intestinal microbial balance. The best evidence for such a protective effect on gut flora stems from the observation that germ-free animals are more susceptible to disease than animals with several intestinal flora. NURMI and RANTALA (1973) showed that young chickens reared under modern husbandry practices and orally pretreated with a diluted faecal solution or mixture of intestinal microorganisms obtained from healthy adult birds, developed resistance to the establishment of *Salmonella infantis* in the intestine. They attributed this increased resistance to competition between the newly-established intestinal flora and the invading pathogen, *S. infantis.* According to GOMEZ-GIL *et al.* (2000), a probiotic ought not to be classified as a biological control agent in the strictest sense, since a probiotic microorganism does not necessarily attack the noxious agent (pathogen) (i.e. a natural enemy of the latter), but it merely prevents damage to the host caused by the pathogen. Such prevention, usually through competition, may produce substances that inhibit the growth or attachment of the harmful microorganism. Neither should probiotics be classified as growth promoters, since their actions are not confined to improved growth but are

also associated with general improvements in health.

Based on these views, biocontrol agents (BCAs) can be considered as natural enemies that kill or repress the growth of pathogenic organisms, and are beneficial or at least not harmful to the plants and animals cultivated.

3. Biocontrol of Bacterial Pathogens in Aquaculture

Following DOPAZO *et al.* (1988), the activity of antibiotic-producing marine bacteria was assayed against bacterial pathogens (*Vibrio, Aeromonas, Pasteurella, Edwardsiella, Yersinia and Pseudomonas*) of fishes with the aim of evaluating the possible use of these marine strains for controlling epizootics in aquaculture. Inhibition tests on solid media showed that the majority of fish bacteria were highly sensitive to the strains tested, only two strains (*Edwardsiella tarda and Pseudomonas aeruginosa*) being resistant to all of the antibiotic-producing strains. TANASOMWANG *et al.* (1998) isolated *Vibrio*-inhibiting marine bacteria from a black-tiger shrimp hatchery. MORIARTY (1998) reported that as a result of using a *Bacillus* species for over 160 days at about 10^4 to 10^5 cells/ml, *Vibrio* numbers, especially those of luminous *Vibrio,* were low in prawn ponds where the *Bacillus* sp. was maintained in the water column. *Vibrio* numbers were also low in the sediments and luminous *Vibrio* absent. Since this report gave no data for fluctuations of *Bacillus* sp. concentrations in the pond, it is unclear whether or not the species added could grow in seawater. One strain of *Alteromonas* showed greater antagonism against fish and shrimp bacterial pathogens than other strains (JAYANTH *et al.,* 2001). In addition, RUIZ *et al.,* (1996) found antagonisms of *Alteromonas* sp. to a large number of bacteria in the aquaculture biotope. The inhibitory effect of *Vibrio alginolyticus* against a *Vibrio harveyi* strain was greater in seawater at 10 ppt. of salinity compared with 20 and 30 ppt. (RUANGPAN *et al.,* 1998). According to RICO-MORA *et al.* (1998), a bacterial strain (SK-05), selected for its active growth in organic-poor substrates and inoculated into a *Skeletonema costatum* culture in late exponential growth, prevented the establishment of *Vibrio alginolyticus,* purposely introduced into the diatom culture. Since SK-05 has no bacteriostatic or antibiotic activity against *V. alginolyticus,* they concluded that it had the effect of competitive exclusion, due to its ability to utilize the exudates of *S. costatum,* which maintained an organic-poor environment within the culture, unsuitable for *Vibrio* growth.

These observations indicate a need for further studies of how individual microbes inhibit or promote fish health and growth, several reports next having indicated beneficial effects to fishes.

JÖBORN *et al.* (1997) reported the production of a growth inhibitor against two common fish pathogens *Vibrio anguillarum* and *Aeromonas salmonicida* by *Carnobacterium* sp., such being demonstrated *in vitro* in mucus and fecal extract. The *Carnobacterium* cells remained viable in the gastrointestinal tract for several days, no detrimental effects on the fish being observed as a result of the presence of the bacterium. SMITH and DAVEY (1993) noted that fluorescent *Pseudomonas* was capable of inhibiting the growth of *Aeromonas salmonicida* in culture media, such inhibition being probably due to the siderophore effect, resulting in competition for free iron. They found that the strain used was also capable of excluding *A. salmonicida* from the fish with stress-inducible infections, and suggested that as the strain did not significantly invade the fish following bath treatment, the effect must have been generated from external sources. Siderophore is a microbial iron (III)-transport agent that sequesters a limited supply of iron (III) and limits its availability to pathogens, ultimately suppressing their growth. Also, as a siderophore effect, GATESOUPE (1997) described the proportion of *Vibrio* sp., dominant in healthy turbot larvae, as being artificially increased in a rotifer enrichment medium and rotifers fed to the larvae. The main purpose of such *Vibrio* enrichment was to improve the resistance of larval turbot challenged by a pathogenic strain of *Vibrio splendidus.* GATESOUPE (1997) concluded that the biocontrol effect of the *Vibrio* sp. used may have been at least partly due to competition for iron with the pathogen.

ANDLID *et al.* (1995) reported 3.8×10^4 to 2.3×10^9 viable yeast cells per gram of intestine or feces in fish. Although the concentration of yeast in their experimental fish tank water never exceeded 10^3 viable cells per milliliter, no indication of fish sickness as a result of the high yeast colonization was recorded during any of the colonization experiments. During the period of their experiments, the concentrations of intestinal aerobic bacteria were lower than the intestinal yeast concentration.

Biocontrol effects and the manner in which biocontrol agents affected the growth of shellfish were reported by NAKAMURA *et al.* (1999). They showed that 12 strains of 51 isolates had inhibitory effects on the growth of 3 vibrios (*V. alginolyticus* and others) tested by a smear technique on an agar plate. One strain, which demonstrated the greater inhibitory effect, had no harmful effects on oysters. The challenge test of *V. alginolyticus* with this bacterium indicated greater than 70% survival of shellfish, while only about 8% survived without the bacterium. This report presents biocontrol agents that promoted the survival rates of shellfish. However, identification of biocontrol agents is still necessary, because of their possible harmful affects on humans.

Several studies have focused on the identification and use of bacteria as biocontrol agents in aquaculture. RIQUELME *et al.* (1996) noted that a culture supernatant of *Alteromonas haloplanktis* stationary-phase cells delayed the growth of pathogens, although the supernatant of the same strain from early and middle log phase growth stages did not negatively affect the growth of pathogenic *Vibrio* spp. Their larval scallop survival experiments showed that preconditioning of larvae with the bacterium for a short time (1 h) was effective in providing larval protection against such *Vibrio* spp. Pre-incubation for 24 h resulted in no significant differences from the control, the most effective result being obtained with the 1-h bath and the addition of the pathogen at 10^3 cells/ml. However, at a concentration of 10^6 cells/ml of the pathogen, the protective effect decreased. This technique, termed "bacterization" in agriculture, wherein bacterial inoculation of seeds or roots leads to changes in plant growth, sometimes yields positive effects and the biological control of some plant pathogens (BROWN, 1974). AUSTIN *et al.* (1995) showed that following the addition of freeze-dried culture supernatant of the BCA, *Vibrio alginolyticus,* to *Vibrio ordalii,* there was a rapid decline of *V. ordalii* numbers (compared to the controls) occurred within 3 h. Following similar treatment, *Aeromonas salmonicida* and *Vibrio anguillarum* counts decreased steadily over 24 h, whereas *Yersinia ruckeri* increased in numbers and did not appear to be adversely affected by the BCA supernatant. These authors also applied the BCA to Atlantic salmon, which led to a reduction in mortality due to *Aeromonas salmonicida,* and to a lesser extent to *Vibrio anguillarum* and *V. ordalii.* GRAM *et al.* (1999) reported that sterile-filtered culture supernatants from iron-limited (0.1 mM) *Pseudomonas fluorescens* inhibited the growth of *Vibrio anguillarum,* whereas sterile-filtered supernatants from iron-replete cultures of *P. fluorescens* did not. *P. fluorescens* inhibited the growth of *V. anguillarum* during culture, independently of iron concentration, when the initial count of the antagonist was 100 to 1,000 times greater than that of the fish pathogen. GRAM *et al.* (1999) also tested the BCA effect *in vivo* by exposing rainbow trout to *Pseudomonas fluorescens* at a density of 10^5 CFU/ml for 5 days before a challenge with *Vibrio anguillarum* at 10^4 to 10^5 CFU/ml for 1 h. Some fish were also exposed to *P. fluorescens* at 10^7 CFU/ml during the 1-h infection. The combined BCA treatment resulted in a 46% reduction in calculated accumulated mortality; accumulated mortality was 25% after 7 days at 12℃ in the BCA-treated fish, compared with 47% in fish not treated with the BCA.

ROBERTSON *et al.* (2000) showed that feeding salmonids with diets containing a probiotic (*Carnobacterium* sp.), being antagonistic against several pathogens, revealed that the strain remained viable in the gastrointestinal tract and that after 14 days of feeding challenge by cohabitation demonstrated its effectiveness in reducing disease caused by *Aeromonas salmonicida, Vibrio ordalii* and *Yersinia ruckeri,* but not *Vibrio anguillarum.* NIKOSKELAINEN *et al.* (2001) administered

Lactobacillus bacterium, *Lactobacillus rhamnosus* at different doses (10^9 and 10^{12} CFU/g–feed) to rainbow trout for 51 days. After sixteen days the fish were challenged with *Aeromonas salmonicida* spp. *salmonicida,* which normally causes furunculosis. However, the administration of *Lactobacillus rhamnosus* resulted in a significant reduction in fish mortality, from 52.6% in the control to 18.9% and 46.3% in the 10^9 CFU/g feed and the 10^{12} CFU/g feed groups, respectively.

GIBSON *et al.* (1998) studied the BCA ability of a bacteriocin–like inhibitory substance–producing *Aeromonas media* strain A199 by assessing its action on the survival of oyster larvae (*Crassostrea gigas*), challenged with *Vibrio tubiashii.* The larvae challenged with *Vibrio* died within 5 days, whereas the presence of the pathogen and BCA strain together did not affect the viability of the larvae over the same time period. The viability of larvae challenged with strain A199 alone was also unaffected. In addition, that BCA exhibited antagonistic activity against a wide range of fish/shellfish pathogens *in vitro.*

Introduction of the spores of *Bacillus* sp. into a culture medium of rotifers, which filtered more than 90% of the spores in 1 h, greatly altered the associated flora of the rotifers. After 5 days of culture, a species of the family Vibrionaceae was dominant in the control rotifers, whereas the spore–fed rotifers had very diverse flora. The mean weight of turbot at day 10 was significantly improved with the spore–fed rotifers, their survival rate also increasing (GATESOUPE, 1991).

Studies of MAEDA (1988), MAEDA and NOGAMI (1989), MAEDA and LIAO (1992), MAEDA *et al.* (1992), NOGAMI and MAEDA (1992), MAEDA and LIAO (1994), NOGAMI *et al.* (1997), MAEDA *et al.* (1997) and MAEDA (1999) have reported biocontrol ability in isolated bacteria, *Thalassobacter utilis* and *Pseudoalteromonas undina,* in promoting the growth of fishes and crustaceans, and inhibiting the growth of pathogens. The use of these bacteria for larval production of *Portunus trituberculatus* (crab), *Penaeus monodon* (prawn), and *Caranx delicatissimus* (fish), not only resulted in high survival rates of all but also repressed diseases caused by bacteria and viruses. In addition, *Thalassobacter utilis* inhibited the growth of pathogenic fungi as well as bacterial pathogens (NOGAMI *et al.,* 1997).

4. Biocontrol of Viral Pathogens

Several viral diseases have had serious implications for the fish rearing industry. Similarly, the culture of penaeid shrimps (*Penaeus monodon* and *P. japonicus*) has been infected by baculo–like viruses. In Taiwan, the production of *P. monodon* decreased from about 90,000 metric tons in 1987 to 30,000 in 1988, dropping further to 20,000 in 1989. SINCE 1993, the *P. japonicus* rearing industry in Japan has been seriously affected by a virus infection, many nursery ponds in the western part of Japan having stopped production. Other viruses exist which are also significant pathogens of finfish. These include infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) (which infect salmon), Hirame rhabdovirus (HIRRV) (flounder), the yellowtail ascites virus (YAV) (yellowtail), striped jack (Sima–Aji) nervous necrosis virus (SJNNV) (mainly striped jack) and an iridovirus (sea bream). All of these can cause serious harm to aquaculture.

In the marine environment, viruses that are obligate parasites of cellular organisms, which are usually specific to certain hosts, are abundant. Virus infections have been found in almost all organisms, although it appears that most of the viruses present in seawater infect bacteria and are responsible for about 10–40% of total bacterial mortality. In addition, the release of dissolved organic matter (DOM) during the lysis of microbes is thought to stimulate the activity of other bacterial components in the water column. Viruses are also involved in genetic transfer and can influence species compositions (FUHRMAN, 2000).

FAURÉ–FREMIET *et al.* (1963) and BERGH *et al.* (1989) reported that the use of direct counting methods with an electron microscope resulted in virus counts that were much higher than those previously reported from natural aquatic environments (based on counts of plaque–forming units using various host bacteria) (SPENCER 1955). According to BERGH *et al.*

Table 1. Biocontrol agents used in aquaculture

Biocontrol agents	Pathogens tested	Fishes reared	Resources
Thalassobacter utilis	*Vibrio anguillarum*	shrimp(*Penaeus monodon*)	Maeda & Nogami(1989)
Pseudoalteromonas undina	IHNV	crab(*Portunus trituberculatus*)	Nogami & Maeda(1992)
	SJNNV	striped jack(*Caranx delicatissimus*)	Maeda & Liao(1994)
	baculo-like virus	sea-bream(*Pagrus major*)	Maeda *et al.*(1997)
	irido virus		Maeda(1999)
Bacillus sp. through rotifers	Vibrionaceae	turbot(*Scophthalmus maximus*)	Gatesoupe(1991)
Pseudomonas sp.	*Aeromonas salmonicida*	brown trout(*Salmo trutta*) Atlantic salmon(*Salmo salar*)	Smith & Davey(1993)
yeast	intestine bacteria	trout(*Salmo gairdneri*)	Andlid *et al.*(1995)
Vibrio alginolyticus	*Vibrio ordalii* *Vibrio anguillarum* *Aeromonas salmonicida*	Atlantic salmon(*Salmo salar*)	Austin *et al.*(1995)
Alteromonas haloplanktis	*Vibrio alginolyticus* *Vibrio anguillarum*	scallops(*Argopecten purpuratus*)	Riquelme *et al.*(1996)
Vibrio sp. through rotifers	*Vibrio splendidus*	turbot(*Scophthalmus maximus*)	Gatesoupe(1997)
Carnobacterium sp.	*Vibrio anguillarum* *Vibrio salmonicida*	rainbow trout(*Oncorhynchus mykiss*) Atlantic salmon(*Salmo salar*)	Jöborn *et al.*(1997)
Thalassobacter utilis	*Haliphthoros* sp. (fungus)	crab(*Portunus trituberculatus*)	Nogami *et al.*(1997)
Aeromonas media	*Vibrio tubiashii*	Pacific oyster(*Crassostrea gigas*)	Gibson *et al.*(1998)
Pseudomonas fluorescens	*Vibrio anguillarum*	rainbow trout(*Oncorhynchus mykiss*)	Gram *et al.*(1999)
bacteria not identified	*Vibrio alginolyticus* *Vibrio tubiashii*	Pacific oyster(*Crassostrea gigas*)	Nakamura *et al.*(1999)
Aeromonas spp.	IHNV	masu salmon(*Oncorhynchus masou*)	Yoshimizu & Ezura(1999)
Vibrio spp.	OMV BF-NNV	barfin flounder(*Verasper moseri*)	
Carnobacterium sp.	*Aeromonas salmonicida* *Vibrio ordalii* *Yersinia ruckeri*	Atlantic salmon(*Salmo salar*) rainbow trout(*Oncorhynchus mykiss*)	Robertson *et al.*(2000)
Lactobacillus rhamnosus	*Aeromonas salmonicida* spp. salmonicida	rainbow trout(*Oncorhynchus mykiss*)	Nikoskelainen *et al.*(2001)
Pseudomonas sp.	*Vibrio harveyi* *Vibrio fluvialis* *Vibrio parahaemolyticus* *Vibrio damsela*	Shrimp(*Penaeus monodon*)	Chythanya *et al.*(2002)
Vibrio spp. *Bacillus* spp.	*Vibrio harveyi*	Shrimp(*Penaeus vannamei*)	Gullian *et al.*(2004)

IHNV: Infectious Hematopoietic Necrosis Virus
SJNNV: Striped Jack Nervous Necrosis Virus
OMV: *Oncorhynchus masou* Virus
BF-NNV: Barfin Flounder Nervous Necrosis Virus

(1989), viral concentrations changed from 10^4 to 10^8 virus particles/ml, which indicates a possible effect of anti-viral microorganisms on the presence of virus particles in seawater. In fact microorganisms in both sea and fresh water commonly inactivate viruses (see below), being responsible for great fluctuations in viral concentrations in the aquatic environment. In addition, water borne viruses have the ability to transfer from one infected organism to another. If anti-virus bacteria dominate the aquatic environment, virus transfer among fish communities could be repressed to a large extent. Based on this concept, anti-viral bacteria are now used in larval rearing procedures in commercial aquaculture (MAEDA *et al.*, 1997; MAEDA, 1999; YOSHIMIZU and EZURA, 1999).

The bacterial strain VKM-124, *Pseudoaltero-*

monas undina, when used in aquaculture, prevented fish larvae from being infected by SJNNV, baculo-like viruses and iridovirus. When added to water at a concentration of about 10^6 cells/ml in rearing containers of *Penaeus* prawn and striped-jack (*Caranx delicatissimus*) larvae, the survival rates of the larvae were much greater with the bacteria than in its absence. In fact, without the addition of the bacteria, all fish and prawn larvae died due to viral infection (MAEDA and LIAO 1994; MAEDA *et al.,* 1997; MAEDA, 1999). According to HERRMANN *et al.* (1974), WARD and ASHLEY (1976), CLIVER and HERRMANN (1972) and HERRMANN and CLIVER (1973), viral inactivation is mainly due to degradation of the viral capsid by proteinases. Thus, biocontrol agents, for example proteinase-producing bacteria, may inhibit the multiplication of viruses among fishes.

According to YOSHIMIZU and EZURA (1999), fish intestinal bacteria, such as *Aeromonas* spp. and *Vibrio* spp., producing anti-viral substances, were isolated from masu salmon (*Onchorhynchus masou*), Japanese flounder (*Pararichthys olivaceus*) and barfin flounder (*Verasper moseri*). The *Aeromonas* strains produced anti-infectious hematopoietic necrosis virus substances, and the *Vibrio* strains showed anti-IHNV, *Oncorhynchus masou* virus (OMV) and barfin flounder Nervous necrosis virus (BF-NNV) activities. When *Aeromonas* spp. strains were mixed with feed pellets and fed to rainbow trout (*O. mykiss*) and masu salmon, the bacteria became dominant in the intestinal microflora and anti-IHNV activity was observed in homogenates of the intestinal contents. The rainbow trout and masu salmon that were fed the *Aeromonas* spp. showed more resistance to the artificial IHNV challenge test. Barfin flounder fed on *Vibrio* sp. strain with *Artemia salina* showed anti-OMV and anti-BF-NNV activities in the intestinal contents.

5. Virus Distribution and Survival in Seawater

Several reports have considered the abundance of viruses (although in some cases, short-term inactivated) in seawater. FAURÉ-FREMIET *et al.* (1963) found that the ciliated protozoan, *Zoothamnium alternas,* was attached to and formed a considerably large community on the body surface of the crab, *Cancer pagurus.* Bacteria attached to the surface of the ciliate showed enormous numbers of bacteriophages (two kinds apparent) inside the cell under an electron microscope examination. BERGH *et al.* (1989) also reported virus counts in the range of 10^4–10^8 virus particles/ml of water using a direct counting method with an electron microscope. Distribution patterns of virus particles in seawater have also been reported by HELDAL and BRATBAK (1991), WEINBAUER *et al.* (1995), BRATBAK *et al.* (1996) and STEWARD *et al.* (1996).

According to GERBA *et al.* (1977), significant concentrations of human viruses occurred in water and sediments of a coastal canal into which secondarily treated sewage was discharged. LABELLE *et al.* (1980) found that viruses existed in greater numbers in sediment than in overlying seawater and SMITH *et al.* (1978) reported that viruses survived for longer periods in sediment, than in overlaying estuarine water. LABELLE and GERBA (1979) studied the adsorption and elution characteristics of several enteroviruses and a rotavirus in estuarine sediments under varying conditions of pH, salinity and presence of soluble organic matter. More than 99% of the added poliovirus, coxsackievirus, echovirus and rotavirus were adsorbed into the sediment. Under similar conditions, some viruses were attached significantly less than the poliovirus. GERBA and SCHAIBERGER (1975a) investigated the loss of viral titers of *Escherichia coli* B bacteriophage in natural seawater without kaolinite and with 500 mg kaolinite/l, in which the virus activity was protected by the presence of kaolinite. Viruses accumulated in sediments near the shore could be easily released into seawater by simple mechanical shaking (FLORA *et al.,* 1975). During feeding, bivalves (oysters, mussels and clams) can accumulate pathogenic human enteric viruses from sewage-polluted seawater. Enteric viruses, such as polio, echo, coxsackie and reo viruses, have been detected in shellfish, field and laboratory studies having indicated that enteric viruses can survive in shellfish for long periods (GERBA and GOYAL, 1978).

METCALF and STILES (1965) reported coxsackie and other enteric viruses from the eastern oyster, *Crassostrea virginica,* which were located in estuarine waters at distances as great as 4 miles from the nearest raw sewage outlet. The virus remained relatively stable within oyster tissues stored at 5℃ for at least 28 days. Of all the tissues examined, the digestive gland showed the greatest retention of the virus, but it was not possible to demonstrate the occurrence of virus multiplication in any of the oyster tissues examined. METCALF and STILES (1965) suggested that the vector potential of oysters resulted from the stability of the virus within oyster tissues following ingestion from environmental seawater. SUZUKI *et al.* (2001) showed that marine birnavirus (MABV), a member of aquabirnavirus, an opportunistic pathogen in eukaryotic marine organisms with a broad host range in wild and cultured fish and shell-fish, was widely distributed in coastal and pelagic seawater as well as in samples of zooplankton collected from the Pacific Ocean.

6. Inactivation of Viruses in Seawater

A number of studies have considered the inactivation of viruses in seawater. PLISSIER and THERRE (1961) stated that poliovirus was inactivated to a significant degree after several weeks in seawater. According to MATOSSIAN and GARABEDIAN (1967), surface seawater was found to inactivate poliovirus type 1 in some six to nine days. However, boiling of seawater or filtration through a Seitz filter removes the virucidal properties of the former. TORANZO and HETRICK (1982) investigated the survival rates of two fish viruses (infectious pancreatic necrosis virus, and infectious hematopoietic necrosis virus) pathogenic to young salmonids and poliovirus type 1 using untreated fresh, estuarine and sea water samples held at 15 and 20℃. The results indicated longer survival of the salmonid viruses than the poliovirus in saline water, whereas in fresh water, the poliovirus was the most stable. They also noted that at 20℃, the inactivation rate for each virus was independent of salt concentrations in both estuarine and seawater samples. BAUDOUY (1976) found that the cyto-infectious power of the infectious pancreatic necrosis virus subsisted for at least 300 days at 4℃, and for 60 days at 14℃ in less highly mineralized water. Comparatively, the virus strength decreased more slowly at 4℃ in more highly mineralized river water. When filtered, the same river water maintained its infectious power better than untreated homologues.

Physical factors in the aquatic environment also affect the activity of viruses. PIETSCH *et al.* (1977) showed that salinity distinctly affected viral survival rates. DENIS *et al.* (1977) investigated the stability of twenty strains of DNA and RNA viruses in natural, heated and synthetic seawater over a period of 400 days. Their studies, under controlled laboratory conditions, indicated temperature as a critical factor affecting viral inactivation in seawater. Differences were noted between viral groups, serotypes, and also between strains of the same serotype. LO *et al.* (1976) also showed that temperature, rather than salinity, was the critical factor affecting viral stability, in that the higher the temperature, the more rapid was the loss of viral infectivity. O'BRIEN and NEWMAN (1977) have reported that inactivation of the viruses was exponential, and the rates of inactivation appearing to be affected principally by water temperature. BERRY and NORTON (1976) investigated the stability of T2 bacteriophages in seawater under laboratory conditions and in the natural waters of a bay, and reported that inactivation was temperature-dependent, being enhanced by sunlight and sewage pollution. WEINBAUER *et al.* (1995) and WEINBAUER *et al.* (1997) subsequently described the role of sunlight in the removal and repair of viruses in seawater.

According to GERBA and SCHAIBERGER (1975b), extensive aggregation, including that of viruses, takes place in artificial seawater. Viral clumps formed in both natural and artificial seawater might be disaggregated by an increase in the amount of organic matter or a decrease in salinity. GERBA and SCHAIBERGER (1975b) have suggested that aggregation might play a role in the initial decline of viral titers in seawater, as well as reducing the number of "infectious foci" present in seawater.

7. Virus Inactivation by Microorganisms in Seawater

MAGNUSSON *et al.* (1966) reported that heating seawater to temperatures above 45℃ for one hour destroyed its virus inactivating capacity. This function of seawater required a NaCl concentration of 0.1 M or higher, although not directly caused by salinity, suggesting that the presence of marine bacteria inactivates viruses which require salt for their growth. TORANZO *et al.* (1982) stated that in estuarine water and sediment, the stability of poliovirus type 1 showed a 2-log reduction in virus titer at 15℃, occurring within 6-7 days in water samples taken from estuarine waters of Rita of Pontevedra and Chesapeake Bay in the Atlantic Ocean. They also indicated that bacterial extracellular products appeared to be involved in the virus-inactivation process, including coxsackie and other enteric viruses. According to LABELLE and GERBA (1980), the time required to inactivate 99% of poliovirus increased from 1.4 days in seawater alone to 6.0 days when the virus was adsorbed into sediment at a relatively non-polluted site. TORANZO *et al.* (1982) indicated that the addition of sediment to natural seawater containing poliovirus increased the length of virus survival to over three times that in seawater alone. Although this effect was not attributed to virus absorption into sediment particles, thereby aiding virus survival in some way, a similar result was not found under sterile conditions, suggesting that the sediment can protect viruses from inactivation by marine microflora. TORANZO *et al.* (1983b) reported that virus-inactivation rates in infectious pancreatic necrosis virus differed significantly in untreated and filtered (or autoclaved) estuarine water samples. In untreated water, the time required for a 90% reduction in IPNV infectivity was only 9 days, whereas it took over 35 days in autoclaved water. IPNV viability was also favored in filter-sterilized water, where it survived nearly four times longer than in untreated estuarine water. Interestingly, the period of the most rapid viral inactivation was correlated with the highest bacterial numbers in untreated water, which suggested that autochthonous microbial flora played an important role in the virus inactivation process. FUJIOKA *et al.* (1980) found that the time for 90% reduction of poliovirus type 1 at 24℃ in seawater in Hawaii ranged from 24 to 48 h, complete inactivation occurring within 72 to 98 h. In fact, their accumulated evidence strongly indicated the presence of virus-inactivating agent(s) of a microbiological nature in both clean and sewage-polluted seawater. Antiviral activity was lost when the seawater samples were subjected to boiling, autoclaving or filtration through a 0.22- or 0.45 μm, but not through a 1.0 μm pore size membrane filter. Other enteric viruses, such as coxsackie virus and echo virus, were also shown to be inactivated in seawater. Before these studies, GUNDERSEN *et al.* (1967) had already reported the effect of marine bacteria in restoring the virus inactivating capacity (VIC) of seawater, depleted of such by heating and filtration. The bacterium responsible for this inactivation was identified as *Vibrio* spp. MAGNUSSON *et al.* (1967) found that *Vibrio marinus* possessed certain antiviral properties. Characteristically, this property of the bacterium was maintained only if the latter was subcultured at a low temperature (4-12℃), whereas the antiviral property disappeared after a number of subcultures at 25℃. KAMEI *et al.* (1987) also reported the presence of anti-virus bacteria in estuarine and seawater. DIREKBUSARAKOM *et al.* (1998) described several strains of *Vibrio* as showing the antiviral activities to IHNV and *Oncorhynchus masou* virus, as measured by plaque reduction rates. On the contrary, SUTTLE and CHEN (1992) suggested that most bacteria were not responsible for the decay of viruses in seawater.

8. Substances which Inactivate Viruses

TORANZO *et al.* (1983a) investigated the mechanism of enterovirus inactivation by marine bacteria using poliovirus type I as a model virus, with strains of *Pseudomonas* and *Vibrio* spp. isolated from the marine environment. Treatment of the virus, with a cell-free filtrate from late log phase bacterial cultures, seemed to produce alterations in the viral capsid, as shown by a reduction in efficiency of adsorption to host cells, increased sensitivity to ribonuclease, and by the release of ribonucleic

acid from the treated virions. In fact, filtration of a ^{14}C-labelled virus sample through 25-nm pore size filters revealed that the majority of the isotope (85-96%) passed through the filters, indicating extensive capsid disruption.

Several reports have indicated that proteases may alter the infectivity of a few enteroviruses. Because many viruses possess a protein capsid, they should be susceptible to at least some proteolytic enzymes. HERRMANN *et al.* (1974) suggested that proteases play a role in inactivating viruses by degrading their protein coat and reported more rapid inactivation of two enteroviruses in a natural lake than in sterile lake water. WARD and ASHLEY (1976) also indicated that the mechanism of inactivation of a virus in sludge involved cleavage of viral proteins, followed by nicking of the encapsulated RNA. CLIVER and HERRMANN (1972) reported that the inactivation mechanism by proteolytic bacteria (notably *Pseudomonas aeruginosa*) could be distinguished from adsorption or aggregation of virus particles because ^{14}C labels from the virus coat protein, but not ^{32}P from the viral nucleic acid, was taken up by the bacterial cells.

HERRMANN and CLIVER (1973) investigated the means by which coxsackievirus type A9 (CA9) was inactivated by proteolytic enzymes. After the reaction of ^{14}C-leucine-labeled CA9 with the protease, only free leucine was liberated, not the infective viral RNA. However further treatment with 1% sodium dodecyl sulfate at pH 7.0 promoted viral RNA release. Sodium dodecyl sulfate treatment of CA9 that was not reacted with the protease did not inactivate the virus or cause viral RNA release. These results demonstrated that the primary means by which protease-sensitive enteroviruses were inactivated was by the degradation of the virus capsid, with subsequent release of viral RNA.

Evidence exists, however, that some viruses are highly resistant to protease action. In fact, LERNER and MIRANDA'S (1968) study of the interactions of a number of hemagglutinating enteroviruses, reovirus type 2 and poliovirus type 1 after treatment with sodium borohydride, several proteases, or carbohydrases, showed that the hemagglutinating activity of virus particles was destroyed by sodium borohydride and certain glycosidases, but was not altered by a number of proteases. MATHEKA *et al.* (1962) have also reported that some enteroviruses from cattle and swine were stable in the presence of protease, but that the coxsackievirus was inactivated by the latter.

Biologically active materials have been reported as inactivating viruses in the marine biotope. EHRESMANN *et al.* (1977) found that ten members of Rhodophyta (algae) from seawater contained substance(s) which caused a greater than 2 log reduction in the infectivity of herpes simplex virus types 1 and 2. In addition, anti-coxsackie B_5 virus activity was detected in extracts of *Constantinea simplex* Satchelt. DEIG *et al.* (1974) reported that extracts from two species of marine red algae, *Cryptosyphonia woodii* and *Farlowia mollis,* specifically inhibited *in vitro* herpes simplex virus replication. GERBER *et al.* (1958) mentioned that extracted polysaccharides from *Gelidium cartilagenium* (seaweed) and carrageenin showed a marked inhibitory effect on the growth of influenza B and mumps viruses. KATHAN (1965) stated that a preparation from crude kelp inhibited bacterial and viral neuraminidases, and also the multiplication of some influenza viruses in embryonated eggs. The inhibitory mechanism of kelp extract might be due to the prevention of penetration of the virus into host cells by direct binding of the virus or by inhibiting the viral enzyme, since the inhibitory effect occurred when eggs were treated with kelp extracts prior to inoculation with the infective virus. RICHARDS *et al.* (1978) tested extracts of two species of marine algae, *Constantinea simplex* and *Farlowia mollis,* for antiviral activity in tissue culture and in experimental infections of mice. Treatment of confluent mouse embryo fibroblast cell mono-layers with either compound before viral inoculation was effective in inhibiting the replication of the herpes simplex virus, types 1 and 2, and vesicular stomatitis virus, but not encephalomyocarditis virus, semliki forest virus, or murine cytomegalovirus.

9. Antagonism of bacteria and microalgae

Burgess *et al.* (1999) isolated over 400 strains of surface-associated bacteria from various

species of seaweed and invertebrates from Scottish coastal waters, and found 35% of them to be producing antimicrobial compounds. This was considered much higher than the free living marine proportion or soil bacteria producing antimicrobial agents. They also reported that many strains which did not normally produce antibiotics could be induced to do so by exposing them to small amounts of live cells, supernatants from other bacterial cultures or certain chemicals.

Gil-TURNES *et al* (1989) reported that embryos of the shrimp *Plalaemon macrodactylus* were remarkably resistant to infection by the fungus *Lagenidium callinectes,* a recognized pathogen of many crustaceans. An *Alteromonas* sp. bacterial strain, consistently isolated from the surface of the embryos, produced 2,3-indolinedione (isatin), a compound that inhibited the pathogenic fungus. When exposed to the fungus, bacteria-free embryos quickly died, whereas similar embryos reinoculated with the bacteria or treated only with 2,3-indolinedione survived. Gil-TURNES and FENICAL (1992) also reported the resistance of American lobster (*Homarus americanus*) embryos to infection by the pathogenic fungus, *Lagenidium callinectes.* The surfaces of healthy lobster embryos were found to be covered almost exclusively by a single, Gram-negative bacterium, which grew in a dense mosaic pattern. The bacterium produced 4-hydroxyphenethyl alcohol (tyrosol), an antibiotic substance known to be produced by terrestrial fungi to inhibit the growth of the pathogenic fungus.

10. Probiotic Effects and Antagonisms of Intestinal Microorganisms of Fish

According to the review paper of OLAFSEN (2001), mucus in the gastrointestinal tract of fishes is known to serve as a source of nutrients, and enhance colonization by serving as an initial attachment site for bacteria or as a matrix for permanent bacterial attachment. Conversely, the mucus layer in some instances may serve as an effective barrier, providing protection against penetration by invading microorganisms. Evidence of a high proportion of bacteria growing as attached forms in the gastrointestinal tract of several larval groups was considered beneficial for both bacteria and host, where the internal bacteria prevent the colonization and proliferation by pathogens.

Accordingly, the gastrointestinal tract of fishes provides a nutrient-rich habitat for microbial growth, most data indicating that like mammals, fishes have an indigenous intestinal microbiota, at least so far as having autochthonous gastrointestinal microorganisms of different composition from these in the surrounding water. Some authors, however, claim that the intestinal biota mostly reflect the feeding and drinking habits of the animal, and are therefore influenced by the external environment. GATESOUPE (1999) asserted that it was unclear whether or not the intestinal microbiota of aquatic animals changed rapidly with the intrusion of microbes from water, but noted that the influence of food has been clearly demonstrated in larval and juvenile fishes, the influence of bacteria introduced via live food organisms being particularly dramatic during first feeding.

According to a review paper by GOMEZ-GIL *et al.* (2000), the ingestion of bacteria by cold-water fishes at the yolk sac stage resulted in the establishment of a primary intestinal microflora, which persisted beyond first feeding. This was followed by a bacterial succession until the adult microflora was established. It is therefore important to add potential probiotics as soon as possible after hatching, in order to effectively colonize the larval gut before the introduction of live food.

GRAM (1993) found that one-third (67 strains) of the total number of bacterial strains isolated from the intestinal gut of fishes inhibited the growth of one or several of six target pathogenic organisms. The inhibitory action was most pronounced among those strains producing siderophores; mediated competition for iron may explain the inhibitory activity of these strains because the addition of iron eliminated the inhibitory activity of two-thirds of the strains tested. On the other hand, the antibacterial action of 21 strains was not completely eliminated by iron supplementation, such possibly being attributable to the production of several different bacterial inhibitors, such as antibiotics and bacteriocins.

Commensal bacteria with inhibitory activity against pathogens have been isolated from the mucosal surfaces of healthy fishes by several researchers (WESTERDAHL *et al.*, 1991; Olsson *et al.*, 1992; BERGH, 1995). WESTERDAHL *et al.* (1991) found that most of the inhibitory bacteria occurred in the rinse and mucus fractions of the gastrointestinal tract. Of the isolates from the gut with an inhibitory effect against *Vibrio anguillarum,* 60% had an inhibitory effect on five other fish-pathogenic *V. anguillarum* serotypes. Inhibitory effects of the isolates were also shown against *Aeromonas salmonicida* and *Aeromonas hydrophila.* SUGITA *et al.* (1998) isolated *Bacillus* sp. from a dragonet (*Callionymus* sp.), which had an inhibitory activity against other bacteria, although they presented no data on the proportion of *Bacillus* spp. among the gut microbial population. As indicated by SAKATA (1990) and ONARHEIM and RAA (1990), *Bacillus* spp. are not dominant intestinal microorganisms in fishes. OLAFSEN (2001) reported that lactic acid bacteria produced growth inhibiting factors that could inhibit various *Vibrio* spp., especially *Vibrio anguillarum.* Although some reports have described the effect of gut microorganisms on fish health, some of the former presented only the composition of bacterial flora (SUGITA *et al.*, 1996). Since some bacterial strain isolates from the gut inhibited fish growth (ROSS and TOTH, 1974), when distributed at high concentrations, the growth of fishes in the presence of bacteria, even if gut isolates, should be assayed before the application of a probiotic or BCAs.

Many investigators have reported that lactic acid bacteria play an important role in the beneficial biological functions of industrial animals. Accordingly the effects of lactic acid bacteria as a growth promoter in fishes have been studied, resulting in commercial probiotics apparently improving the dietary value of rotifers for flatfish larvae (GATESOUPE *et al.*, 1989). ROBERTSON *et al.* (2000) reported that feeding salmonids with diets containing a probiotic (*Carnobacterium* sp.), which showed antagonism against several pathogens, revealed the viability of an isolate of *Carnobacterium* sp. in the gastrointestinal tract. NIKOSKELAINEN *et al.* (2001) administered *Lactobacillus rhamnosus* at different doses to rainbow trout before the fish were exposed to *Aeromonas salmonicida* ssp. *salmonicida,* thereby reducing fish mortality significantly. This effect could be attributed to the immune system improvement of the fish, the stimulation of the immune system of terrestrial animals by lactic acid bacteria having already been demonstrated. However, in spite of their beneficial effects, lactic acid bacteria formed a minor component of the adherent intestinal microflora of salmonid fry in seawater (OLAFSEN, 2001).

On the other hand, ROSS and TOTH (1974) reported that pathological conditions of rainbow trout were associated with a species of *Lactobacillus.* RINGØ and GATESOUPE (1998) also stated in their review paper that pathogenic lactic acid bacteria, such as *Streptococcus, Enterococcus, Lactobacillus, Carnobacterium* and *Lactococcus* spp., have been detected in the ascites, kidneys, livers, hearts and spleens of fishes.

11. Prebiotics

GIBSON and ROBERFROID (1995) have proposed that prebiotics are significant for establishing intestinal flora. Probiotics have been used to change the composition of colonic microbiots, but the possibility of such changes being transient has limited the implantation of exogenous bacteria. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon, thus attempting to improve the health of the host. Nondigestible oligosaccharides in general, and fructooligosaccharides in particular, are types of prebiotics, having been shown to stimulate the growth of endogenous bifidobacteria, which after a short feeding period of these materials became predominant in human feces, for example.

RINGØ (1993) showed that the total counts of viable colonies in the feces of Arctic charr fed linoleic acid supplemented diet, were higher than in fish fed an unsupplemented diet. The Gram-positive bacteria species (*Lactobacillus* and coryneforms) found in the faeces of fish fed the unsupplemented diet were not present

in feces of the linoleic acid group, linoleic acid having a stimulatory effect on the growth of *Aeromonas* sp., *Pseudomonas* sp. and *Vibrio* sp., whereas the growth of *Lactobacillus* sp. was inhibited. However, the role of these transformed flora in fish fed with linoleic acid has not yet been clearly demonstrated.

12. Conclusions

More than million microorganisms per milliliter inhabit the aquaculture environment and affect each other, both through the substances they produce and emit, as well as through the various ways they come into contact with one another (EDDY and JONES, 2002). These microorganisms in an aquaculture environment cannot be eliminated by sterilization with ultraviolet radiation or ozone treatment, or even by filtration. Bacterial counts decrease with these treatments, but quickly recover to their original levels because microorganisms abound in the surrounding environment. In the case of sterilization using drugs, the number of drug-resistant microorganisms increases in water to levels much higher than those in non-sterilized water, because the chemicals destroy the microbial interactions of the antagonists. In fact, the growth rates of the specific microorganisms increase without antagonists. Furthermore, no one can foresee which bacterial species may occupy the niche vacated by these treatments. Throughout the world, many fish diseases are spreading in aquaculture systems as a result of pathogens that dominate the microbial community. This in turn has resulted in more chemicals used and further increases in pathogens (ABDELZAHER and ELNAGHY, 1998; DIJKSTERHUIS *et al.*, 1999). As described in this review, a number of reports have been published on the inactivation of virus infectivity in seawater, and the presence of anti-virus microorganisms. The growth of these anti-virus bacteria should be almost certainly repressed with antibiotics.

Biocontrol agents (BCAs) that promote fish growth as well as inhibiting pathogens, belong to a variety of bacterial genera that include *Pseudomonas, Vibrio, Pseudoalteromonas, Alteromonas, Carnobacterium, Lactobacillus, Aeromonas* and *Thalassobacter*. The antagonistic effect of these BCAs on pathogens is due to the production of bacteriocins, siderophores and enzymes, as well as niche exclusion. Several reports have suggested that niche exclusion among microbial populations can possibly eliminate pathogens, even if the microorganisms used do not exert antimicrobial effects on the pathogens directly. In addition, certain bacteria have been found to repress viral pathogens in aquaculture, but the virus inactivation processes generated by these bacteria have not been elucidated, although several reports cited have suggested degradation of the viral capsid by proteases.

Due to the risks of chemical intervention in aquaculture and concerns about the safety of aquacultural fishes as food sources, the use of drugs as a mainstream response to pathogens is likely to be increasingly constrained by regulations, and consumer and environmental pressures. Thus, alternative methods for disease management such as biocontrol should remain as one of the key strategies. In addition, an increasing need for biocontrol agents seems highly likely, owing to global climate changes combined with increasing free trade most likely leading to an expansion of a range of diseases, from tropical and subtropical to northern countries.

MALAKOFF (1999) described biocontrol as "fighting fire with fire," in which exotic enemies that have been released against pathogens become harmful and disturb the natural ecosystem, and HOWARTH (2000) warned that the administration of non-indigenous agents could potentially result in an irreversible impact on the natural environment, although most of the environmental harm caused by native agents would normally be reversible. In similar vein, WRATTEN and GURR (2000) noted that after a first target of pathogens was reduced in number, further biological control programs would have to be developed in a potentially never ending cycle of adventive organisms exploiting anthropogenic or natural resources, forming a "biological control treadmill".

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