DISTRIBUTION OF USEFUL AND HARMFUL MICROORGANISMS IN SHRIMP AQUACULTURE WATER IN TIEN HAI COASTAL OF THAI BINH PROVINCE

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ABSTRACT

Microorganisms in shrimp aquaculture water are very diversity and variety with useful and pathogenic bacteria groups such as **Bacillus megatherium, Aeromonas hydrophila, Aeromonas salmonicida, Pseudomonas aeruginosa** *and* **Vibrio alginoliticus.** *During shrimp aquaculture, nitrifying and denitrifying bacteria raised 100 to 1000 times. Fermentative bacteria increase up to 10^6 - 10^7 CFU/ml in hatch shrimp ponds. In the pond where shrimp dead, amount of aerobic bacteria decreased remarkably, 1000 times, compared with where shrimp healthy, even though SRB increased significantly, 10^6 CFU/g. The useful microorganisms dominated in healthy shrimp pond (***Bacillus, Pseudomonas***). In contrast, the pathogenic bacteria (***Aeromonas, Vibrio***) used to find in the pond that observed dead shrimp*

INTRODUCTION

Recently, our aquaculture has been developing and becoming one of the most important economic fields in Vietnam. The total of export turnover value of this field in 2002 is more than 2 billion USD. The shrimp area is increasing quickly, making up more than 90% of marine aquaculture. However, this increase is spontaneous; people do not pay attention to ecology factors. Hence, the environment is polluted and regressive and the ecology balance is broken down. As a result, shrimp productivity is decreasing and the productivity of many ponds is less than 50kg/ha/year.

In cultivation - aquaculture methods breed and water source are two significant factors. Therefore, we should estimate the number and component of each microorganism population in input shrimp aquaculture water to choose suitable control procedures, increasing the activity of useful microorganism group, restricting the number and effect of pathogenic microorganism group, distributing on the culturing shrimp quality, reinforcing culturing productivity as well as protecting the environment.

MATERIALS AND METHODS

Materials

- Collecting water and sediment samples from shrimp culturing ponds in Nam Cuong coastal of Thai Binh province.
- Selective media: API RP 38 medium for aerobic microorganism; Basruda medium for nitrifying bacteria, Giltai medium for denitrifying bacteria, Postgate B for sulfate - reducing bacteria, MRS medium for fermentative bacteria.

Methods

- Counting the number of bacteria with limited diluting method and Koch method
- Defining the existence of nitrifying bacteria with diphenylalamin.
- Observing colony, cell morphology and the Gram reaction
- Classifying microorganism by biochemical tests API 50CHB and API 20NE

Distribution of useful and harmful microorganism in shrimp aquaculture water before placing breed shrimp into water

Collecting 6 samples Bn, Bb, Nn, Nb, Gn, Gb in three points $A_0$1, $A_0$2, and A03 before placing breed shrimp into water 2 - 4 days. The analyzing result is in table 1.

The component of microorganism in water samples at this time is quietly diversity. Generally, in sediment samples, the number of microorganism is more variable and abundant than in water samples. In all three sediment samples, all six analyzing bacteria groups are present, while in three water samples; only three bacteria groups are present.

Aerobic and fermentative bacteria are present in all samples with a high number. This means that, organic compound is strongly broken. This is a good sign for shrimp aquaculture. Sulfate-reducing bacteria are only present in sediment samples with permitting amount (10^4-10^5 in sediment). The component and number of microorganism groups in three points $A_0$1, $A_0$2, $A_0$3 are quietly equal, but sulfate - reducing bacteria in $A_0$2 point is higher than the others.

Table 1. Microorganism in shrimp aquaculture samples before placing breed shrimp into water

Pond	Coordination	Sample	Level	pH	Eh (mV)	NaCl (‰)	The number of bacteria (CFU/ml (g))					
							Aerobic	NH_4	NO_2	NO_3	Fermentative	SRB
$A_0$1	20° 22'091	Bn	Water	7,38	160	15	10^6	0	0	10^3	10^4	0
	106°33'797	Bb	Sediment				10^7	10^1	10^1	10^5	10^5	10^2
$A_0$2	20° 21'886	Nn	Water	7,2	131	16	10^6	0	0	10^4	10^4	0
	106°33'824	Nb	Sediment				10^6	10^1	10^1	10^4	10^5	10^3
$A_0$3	20° 22'085	Gn	Water	7,3	170	17	10^6	0	0	10^4	10^4	0
	106°34'095	Gb	Sediment				10^6	10^1	10^1	10^4	10^5	10^2

Distribution of useful and harmful microorganisms in aquaculture after culturing 4 weeks

After culturing 4 weeks, beside 3 points $A_0$1, $A_0$2, $A_0$3, collecting samples in 1 new point $A_0$4. The characteristics of these samples are remarkably different: In points $A_0$2 and $A_0$4, shrimp is weakly, there is unusual sign on shrimp's body and died gradually; at points $A_0$1 and $A_0$3, shrimp is healthful and grows strongly. The analyzing result about microorganism in shrimp aquaculture samples in the second collecting sample is in the table 2.

After culturing 1 month, the component and number of each microorganism group in all samples tend to increase. In all four water samples, all representations of bacteria are present. The number of denitrifying and fermentative bacteria increases from 10^3 - 10^4 cell/ml (before placing shrimp) to 10^5 - 10^6 cell/ml. The number of useful nitrifying bacteria and pathogenic sulfate - reducing bacteria in sediment samples increases, too.

Basing on the data in table 2, the number of sulfate - reducing bacteria in water and sediment samples in $A_0$2, $A_0$4 points is clearly higher than in $A_0$1, $A_0$3. According to Lai Thuy Hien *et. al.*, if the number of sulfate - reducing bacteria in water sample is more than 10^2 - 10^3 cell/ml and in sediment sample is more than 10^6 cell/g, there is polluted. Therefore, $A_0$2 and $A_0$4 can be polluted. Moreover, the Eh value in these two points is negative. This means that sulfate - reducing bacteria (harmful anaerobic bacteria) is higher than oxidative bacteria (useful aerobic bacteria). On the other hand, in $A_0$1 and $A_0$3, the Eh value is positive; the number of aerobic

bacteria (totally aerobic and nitrifying bacteria) is higher than anaerobic bacteria (denitrifying and sulfate - reducing bacteria).

In general, the environment of shrimp aquaculture in two points A_02 and A_04 begins polluted and shrimp is weakly and died gradually. In A_01 and A_03 points, shrimp is healthful and grows strongly. Basing on the experiment and practice, we concluded that, if there are a lot of pathogenic bacteria (such as sulfate - reducing bacteria) in shrimp culturing water, it is very harmful and it becomes an agent that directly or indirectly affects shrimp productivity.

Table 2. Microorganism in shrimp aquaculture water samples after 4-week culturing

Pond	Sample	Level	Sample characteristics	pH	Eh (mV)	NaCl (‰)	The number of bacteria (CFU/ml (g))					
							Aerobic	NH_4	NO_2	NO_3	Fermentative	SRB
A_01	Bn	Surface water	Ponds appear no dead shrimp	7,4	120	16	10^6	10^1	10^2	10^5	10^4	0
	Bb	Sediment					10^6	10^2	10^3	10^6	10^6	10^3
A_02	Nn	Surface water	Ponds Appear dead shrimp	7,0	70	15	10^5	10^1	10^2	10^5	10^5	10^3
	Nb	Sediment					10^3	10^3	10^2	10^6	10^6	10^6
A_03	Gn	Surface water	Ponds Appear no dead shrimp	7,3	130	17	10^6	10^1	10^2	10^5	10^5	10^1
	Gb	Sediment					10^6	10^2	10^2	10^6	10^6	10^3
A_04	Dn	Surface water	Ponds appear dead shrimp	6,9	80	15	10^4	10^3	10^2	10^5	10^6	10^3
	Db	Sediment					10^4	10^2	10^2	10^6	10^6	10^6

Distribution of useful and harmful microorganisms in water after 15 week culturing

In the following days, dead shrimp water in Ao2 and Ao4 ponds were treated by chloramphenicol. Ao4 pond was consequently added probiotic Pond-Clear. Microbial analysis result after 15 week culturing was shown in the table 3.

Table 3. Microorganisms in shrimp aquaculturing water after 15 week culturing

Pond	Sample	Layer	pH	Eh (mV)	NaCl (‰)	Bacteria (CFU/ml (g))					
						2.1.2.1. Aerobic	NH_4	NO_2	NO_3	Fer.	SRB
A_01	Bn	Water	8.2	127	16	10^6	10^1	10^1	10^5	10^6	10^1
	Bb	Deposit				10^6	10^2	10^1	10^6	10^7	10^5
A_02	Nn	Water	8.1	20	15	10^6	10^1	10^1	10^4	10^5	10^2
	Nb	Deposit				10^6	10^2	10^2	10^5	10^6	10^6
A_03	Gn	Water	8.0	117	15	10^7	10^1	10^2	10^5	10^6	10^1
	Gb	Deposit				10^6	10^2	10^3	10^6	10^6	10^5
A_04	Dn	Water	8.1	80	16	10^6	10^1	10^2	10^4	10^5	10^1
	Db	Deposit				10^7	10^2	10^2	10^6	10^6	10^4

Compared to other ponds, although total of bacteria decreased in pond Ao2, number of SRB was still high. Otherwise, distribution of every type of microbes was relative equal. Useful bacteria (carbon-degrading and nitrogenous-converting bacteria) predominated over SRB.

Characteristics of some isolates

After sampling, we isolated and selected specific strains of every sample. The result was shown shortly.

Table 4. Characteristics of some strains isolated from shrimp culturing pond

Sample	Strain	Colony	Cell	Identification
Bb	NC-b1	Yellowy, flat, wrinkled, dry, Serrated edge, 7-8 mm	G (+), long big rod	*Bacillus megatherium*
Nn	NC-n3	White, slightly convex, smooth, tidy edge, 3-4 mm	G (-), long small rod	*Aeromonas hydrophila*
Nb	NC-b3	Brown yellow, spread, flat, serrated edge, > 10 mm	G (-), short big rod	*Vibrio alginoliticus*
Gn	NC-n5	Yellow green, spread, rough, secrete green pigment, > 10 mm	G (-), short big rod, oval	*Pseudomonas aeruginosa*
Dn	NC-n7	Brown yellow, flat, smooth, spread, serrated edge, > 10 mm	G (-), long smal rod	*Aeromonas salmonicida*

Among five isolated strains, three pathogenic bacteria *V. alginolyticus, A. salmonicida* and *A. hydrophila* are the most worth attention in shrimp culturing [10]. *Pseudomonas* sp. was usually used in probiotic because of their protein breakdown and denitrifying ability. Especially, *B. megatherium* is a very useful species because it not only absorbs organic compounds but also eliminate pathogenic bacteria such as Vibrio and Aeromonas [7, 9]. Unfortunately, we isolated pathogenic bacteria from dead shrimp ponds, Ao2 and Ao4. We should check more whether they kill shrimp or not. Shrimp was only killed when together three agent sterile, disease-germ and resistance affect badly on. However, useful bacteria *B. megatherium* and *P. aeruginosa* were isolated from healthy shrimp ponds Ao1 and Ao3.

The results showed that distribution of microorganisms in shrimp aquaculture water was very important, even though we should study more why shrimp die. Harmful bacteria may be directed or undirected factor reducing shrimp productivity. In contrast, useful bacteria make up safe environment for shrimp growth, resulting in productivity increase.

CONCLUSION

From 30 samples taken from typical shrimp aquaculture ponds in Tien Hai coast of Thai Binh, analysis results showed that:

- Water had both useful and harmful microorganisms at the same time such as *Bacillus megatherium, Nitrosomonas* sp., *Nitrobacter* sp., *Aeromonas hydrophila, Pseudomonas aeruginosa* and *Vibrio alginoliticus*.
- Number of both types of microorganisms was changed during aquaculturing. NH_4, NO_2 and NO_3 converting bacteria increased 100 to 1000 folds; fermentative bacteria were up to 10^6-10^7 CFU/ml in the hatch pond. In the dead shrimp pond, number of aerobic bacteria decreased 1000 times, that of SRB remarked increased, 10^6 CFU/g mud.
- In the healthy shrimp ponds, useful microorganisms predominated, *Bacillus, Nitrosomonas, Nitrobacter, and Pseudomonas*. Otherwise, in the dead shrimp ponds, there were many pathogenic bacteria (*Vibrio, Aeromonas*).

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