**PROWASO\_Va**

**SOIL + WATER PROBIOTIC specific for *Litopenaeus vannamei***

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**INTRODUCTION:**

Bad Soil parameters like soil pH, Pond Bottom pollution, Presence of Heavy Metals in the soil, Bound Nutrients like Phosphorous in the soil , Presence of Pollutants, Presence of Pathogens, Presence of obnoxious Gases like H2S, Ammonia will affect the survival and growth of the inhabitant.

Bad water parameters like pH, Salinity, Hardness, Suspended Solids, Dissolved Solids, Turbidity, Algal Blooms, Heavy Metals, Pesticides, Lack of Available N, P and K, Lack of Available Minerals, Lack of D O , Presence of Pollutants, Presence of Pathogens, presence of hazardous gases etc will hamper the growth and survival of the inhabitant.

PROWASO\_Va is a Powder with well balanced beneficial microbes to take care of the above problems arising to the soil and pond bottom containing the dead animals, fecal matter, wasted feed and harmful bottom algae, and to the water borne diseases and problems.

In order to withstand the high stocking densities in *Litopenaeus vannamei* production (hatcheries and pond grow-out) and related stress situations, directly-fed probiotics and water medium probiotics are a promising solution to have a sustained *penaeus* *vannamei culture* by improving survival rate, enhancing immunity, improving FCR and stimulating the body weight gain of the *vannamei.*

**CITATIONS:**

This paper studied the effects of probiotics on the sediment of Penaeus vannamei pond during 117 days of culture period. The results showed that probiotics application significantly decreased the concentrations of total nitrogen, total phosphorous, and sulfide in sediment, but no significant difference was observed in total plate count (TPC) of microbes between treated and control ponds. The final average presumptive vibrio count (PVC) of treated pond sediment (3.65 x 10(3) cfu x g(-1)) was significantly lower than that of the control (1.16 x 10(5) cfu x g(-1)), while the average number of BS (Bacillus), AB (ammonifying bacteria), PSOB (presumptive sulphur oxidizing bacteria) and SRB (sulphur reducing bacteria) in treated pond sediment was higher than that of the control. These data showed that probiotics could decrease the nutrients (nitrogen, phosphate and sulfur) accumulation and improve the composition of bacterial populations in pond sediment, and thus, supply a good sediment environment for the healthily culture of the shrimp.

## (Yanbo Wang, Longying Zha, Zirong Xu; Effects of probiotics on Penaeus vannamei pond sediments; Ying yong sheng tai xue bao; 01/10/2006; 17(9):1765-7.)

The effect of probiotic, *B. coagulans* SC8168, as water additive on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities was investigated at ontogenetic stages (Z3, M3, PL1–2 and PL7–8). Twelve tanks with three replicates for each treatment group and control group were used. The treatments consisted of three SC8168 levels at an initial concentration of 1.0 × 105 cfu ml− 1 (T-1), 5.0 × 105 cfu ml− 1 (T-2) and 1.0 × 106 cfu ml− 1 (T-3) and one control (without any probiotic), and were conducted every day. Addition of the probiotic significantly increased survival rate (*P* < 0.05) for all treatments over controls. However, no significant difference was found between T-2 and T-3. At early larval stages (Z3 and M3), protease activity in shrimp was not significantly different among probiotic treatments and control. At the subsequent ontogenetic stages (PL1–2 and PL7–8), the highest protease activity was observed in T-2 and there was a significant difference (*P* < 0.05) between the treatment and the control. Similar results were observed in T-3 at PL7–8 stage (*P* < 0.05). Amylase activity in T-2 at Z3, M3, PL1–2 and PL7–8 stages was significantly higher (*P* < 0.05) than that in the control. The amylase activity was also increased significantly (*P* < 0.05) in T-3 than the control except the M3 stage. As for the lipase activity, assays showed a significant difference (*P* < 0.05) in groups treated with SC8168 as compared with the control except the initial stage (Z3). However, a concentration response of probiotic strains in T-1, T-2 and T-3 was not observed in the present research. The results from this study suggest that *B. coagulans* SC8168 supplemented at a certain concentration could significantly increase survival rate and some digestive enzyme activities of *P. vannamei* larvae.

(**Xu-xia Zhou, Yan-bo Wang and Wei-fen Li;** Effect of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities; [Aquaculture](http://www.sciencedirect.com/science/journal/00448486)
[Volume 287, Issues 3-4](http://www.sciencedirect.com/science?_ob=PublicationURL&_tockey=%23TOC%234972%232009%23997129996%23843058%23FLA%23&_cdi=4972&_pubType=J&view=c&_auth=y&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=be92bd6dd9682f2673e35cbf448c8418), 18 February 2009, Pages 349-353)

The effectiveness on water quality, population density of bacteria, and shrimp production in ponds treated with commercial probiotics was tested in Penaeus vannamei ponds in Hai-yan, China. Six ponds with replicates for treatment and control were used. Results showed that the probiotics could improve the population density of beneficial bacterial flora, reduce concentrations of nitrogen and phosphorus, and increase yields of shrimp. The average counts of Bacillus sp., ammonifying bacteria, and protein mineralizing bacteria were found to be significantly higher in treated ponds compared to control ponds (P < 0.05). In control ponds, an increase in presumptive vibrios was observed and the average density was up to 2.09 × 103 cfu/mL, whereas that was only 4.37 × 102 cfu/mL in treated ponds (P < 0.05). The use of probiotics also significantly increased dissolved oxygen (P < 0.05) and reduced dissolved reactive-phosphorus, total inorganic nitrogen and chemical oxygen demand (P < 0.05). An average of 8215±265 kg shrimp/ha was obtained in treated ponds with a feed conversion ratio (FCR) of 1.13±0.05 and survival rate of 81.00±6.25% compared with 4985±503 kg shrimp/ha, 1.35±0.12 and 48.67±3.51%, respectively, in control ponds. This indicates that the addition of the commercial probiotics had a noticeable influence on water quality of shrimp ponds and shrimp production.

# [[Wang Yan-Bo](http://www.refdoc.fr/?traduire=en&FormRechercher=submit&FormRechercher_Txt_Recherche_name_attr=auteursNom:%20%28Wang%29), [Xu Zi-Rong](http://www.refdoc.fr/?traduire=en&FormRechercher=submit&FormRechercher_Txt_Recherche_name_attr=auteursNom:%20%28Xu%29), [Xia Mei-Sheng](http://www.refdoc.fr/?traduire=en&FormRechercher=submit&FormRechercher_Txt_Recherche_name_attr=auteursNom:%20%28Xia%29); The effectiveness of commercial probiotics in northern white shrimp Penaeus vannamei ponds; *Fisheries Science*; 2005, vol. 71, no5, pp. 1036-1041 -6 pages]

Effects of *Bacillus subtilis* E20 isolated from fermented soybean on immune parameters and the disease resistance of the white shrimp (*Litopenaeus vannamei*) after 98 days of *B. subtilis* E20 feeding were evaluated in this study. Shrimp fed *B. subtilis* E20-containing diets at concentrations of 106 (E206), 107 (E207), and 108 (E208) cfu kg−1, respectively, had significantly increased survival rates of 13.3%, 16.7%, and 20%, compared to the control (fed no probiotic) after being challenged with *Vibrio alginolyticus*. There were no significant differences in the total hemocyte count, respiratory burst, or superoxide dismutase glutathione peroxidase among all treatments. Shrimp fed a higher concentration of the probiotic (E208) exhibited significant increases in phenoloxidase activity, phagocytic activity, and clearance efficiency compared to control shrimp. In addition, *B. subtilis* E20 showed a weaker inhibitory effect against the growth of *Aeromona hydrophila* with around a 0.3-cm inhibitory zone, but showed no inhibitory effects against other selected pathogens, such as white shrimp pathogens: *V. alginolyticus* and *Vibrio vulnificus*. These results suggest that the increased resistance of shrimp after *B. subtilis* E20 consumption occurs through immune modifications, such as increases in phenoloxidase activity, phagocytic activity, and clearance efficiency against *V. alginolyticus*.

(Deng-Yu Tseng, Pei-Lin Ho, Sung-Yan Huang, Sheng-Chi Cheng, Ya-Li Shiu, Chiu-Shia Chiu,and Chun-Hung Liu; Enhancement of immunity and disease resistance in the white shrimp, Litopenaeus vannamei, by the probiotic, Bacillus subtilis E20; Fish & Shellfish Immunology; Volume 26, Issue 2, February 2009, Pages 339-344)

A study focused on the use of putative bacteria as probiotics to reduce nutritional and disease problems in aquaculture industry was carried out. This study was conducted in two experiments to investigate the putative bacteria flora as probiotics (isolated from Macrobrachium rosenbergii) for enhancement of growth and survival of L. vannamei in duration of 2007 to 2008 at University Putra Malaysia. In the first experiment, a feeding trial was carried out to investigate the potential probiotic properties of Bacillus subtilis isolated from M. rosenbergii on L. vannamei. Putative B. subtilis bacterium was added to commercial shrimp feed as a probiotic. Four types of diets were prepared by mixing the commercial pellet shrimp feed with; i) B. subtilis (T1), ii) mixture of B. subtilis and a commercial probiotic (T2), iii) commercial probiotic as positive control (T3), and iv) an un-supplemented feed as negative control (T4). After 60 days the shrimps fed diet mixed with B. subtilis showed the highest survival rate 75.5± 4.62 % and the greatest yield 190.00± 13.13 g and also there were significant differences (P< 0.05) for bacterial count between T1 and the other treated groups. It was found that, feed treated with B. subtilis appeared to enhance growth and survival rate of L. vannamei at concentration of 1010 CFU/g. Another experiment was carried out to investigate the potential probiotic-ability of B. subtilis to combat with the L. vannamei disease problems. After 60 days of culture, shrimps were challenged by immersion method to V. parahaemolyticus (107 CFU/ml). Four treatment groups were presented in this experiment which were; i) T1- Shrimps treated with B. subtilis in the first experiment were challenged with V. parahaemolyticus, ii) T2- Shrimps treated with mixture of B. subtilis and commercial probiotic in the first experiment were challenged with V. parahaemolyticus, iii) T3- Shrimps treated with unaltered diet were challenged with V. parahaemolyticus as negative control group, and iv) T4- Shrimps treated with commercial probiotic diet were challenged with V. parahaemolyticus as positive control group. After 15 days of the challenge test, there were no significant differences in survival rate between treatment and control groups. There was no significant mortality or disease symptoms due to infection pathogen, and survival rate for all of treatment and control groups was 100%. Another study was carried out to confirm whether V. parahaemolyticus is a pathogen. One hundred shrimp (a new group) with the same size and the same age of shrimps were prepared to confirm the pathogenicity of V. parahaemolyticus. Survival rate after 10 days was 57% due to existing mortality from V. parahaemolyticus. To find the reason of non-mortality of the negative control group during the challenge test with V. parahaemolyticus, bacteria were isolated from digestive tract, muscles and body surface of negative control group, based on morphological observation. Forty three kinds of bacteria were isolated. From these isolated bacteria, 30% were gram positive bacteria, 30% were Pseudomonas spp. and 40% were Enterobacteriacea. Antagonism tests were put for isolated Pseudomonas spp. by the cross-streak method with three pathogens; V. parahaemolyticus, V. alginolyticus, and V. cholerae. Results of antagonist test for four isolated Pseudomonas spp. bacteria showed perfect antagonistic activity against the three pathogens. Perhaps the reason for no observed mortality during the challenge test was due to availability of these natural microflora bacteria (Pseudomonas spp.) inside the body of shrimps, and perhaps they had an inhibitory role against V. parahaemolyticus. Interestingly, there was no Vibrionaceae bacteria found in the shrimps' bodies however a count of 5.5 x 107 CFU/ml of Vibrio bacteria was found from the culture water. It could be possible that, the Pseudomonas spp. from the control group (as a natural micro flora) and B. subtilis for treatment groups played the inhibitory roles against pathogen bacteria or Vibriosis, by action of competitive exclusion or adhesion site

**(**Far, Hadi Zokaei (2009); Effects of Probiotics on the Growth and Survival of Whiteleg Shrimp (Litopenaeus Vannamei) and their Inhibitory Roles against Vibrio Parahaemolyticus. Masters thesis, Universiti Putra Malaysia.)

**CONTENTS AND MODE OF ACTION:**

There are several Modes of Action of Probiotics like

* Antiviral effects
* Elimination of Pathogens by Competition for nutrients
* Elimination of Pathogens by Competition for site attachment
* Digestion enhancement by secretion of Enzymes
* Improvement of quality of Feed Intake by enriching the same with Amino Acids, degrading Toxins, improving the palatability etc.
* Modulation of non-specific immune responses
* Production of antimicrobial compounds
* Water quality improvement like pH, Hardness, Turbidity, Heavy Metals, Obnoxious gases

*Acetobacter Xylinum Produces Acetic Acid and Nucleotides*

Activated Carbon present binds the Harmful Gases and also traps Toxins and Virus.

Activated Hydrated Sodium Aluminum Silicate also entraps the obnoxious Gases and also softens the water by improving CEC.

*B. Nitrobacter* Converts HNO2 into HNO3. (Nitrite into nitrate); Obligates autotrophs

*B. Nitrosomonas* Obligates autotrophs; Solubilizes Phosphorous; Converts NH3 into HNO2.

*B. megaterium* solubilises Phosphorous in higher pH of the medium.

*B. POLYMIXA* produces polymixin, a polypeptide antibiotic, which possess the ability to damage cell membrane structure; Possesses unusual characteristic to fix nitrogen under anaerobic conditions; Solubilises Phosphorous; Asymbiotic Nitrogen fixer; Gram negative; Produces 2,3 Butaned.

*B subtilis* produces amino acids; Gram positive; Thermoduric.; Solubilises Phosphorous; Degrades proteins (Proteolytic) and Carbon (amylolytic); Produces amylases and protease enzymes; Used to modify starches; Can remain active in excreta resulting in less odor, faster decomposition, and in reduction of solids; Produces bacitracin, which interferes with regeneration of the monophosphate form of bactoprenol from the pyrophosphate form.

*De Sulfo Vibrio desulfuricans* produces stable hydrogenase; Useful to remove iron from solutions, metal sulfide formations; Utilises Sulphates and releases H2S.

Enzymes present degrade all the waste materials in the water and at the pond bottom.

Humic substances present also help to soften the water.

*L lactis* reduces the ability of pathogens to grow and cause infection; Especially effective against Listeria monorytogenes, which causes severe food poisoning.

*L acidophilus* produces extremely effective natural antibiotic substances that can inhibit 11 known disease causing bacteria; inhibit yeast infections; Helps in cases of chronic constipation and diarrhea by replacing undesirable intestinal organisms; Helps in the cases of food poisoning; Lowers levels of LDL Cholesterol; Found to alleviate intestinal disorders, principle being that the ingestion of large numbers of the lactobacilli may result in replacement of undesirable intestinal organisms by harmless and beneficial organisms, Aids in nutrient uptake; Fights Candida overgrowth; Controls effectively *E Coli and Staphylococcus aureus.*

*Lactobacillus reutri* effective in enhancing the growth and development of animal; produce reuterin, an antimicrobial agent which inhibits growth of other intestinal pathogenic microbes such as *Salmonella, Listeria, Escherichia*; effectively counteract weight losses caused by disease associated stress and competitively reduce *Salmonella*; Reduced variability in body weights of the animals; Controls *Cryptosporidium parvum* infection; Beneficial in treatment of watery diarrhea.

Organic Acids Present will keep the pH of the water medium slightly acidic so that pathogens does not survive.

*Pediococcus acidilactici* is known to prevent colonization of the small intestine by pathogens like *Shigella, Salmonella, Clostridium* *difficile* and *Escherichia coli* among small animals. It is a probiotic bacterium that presents positive effects on the balance and the role of the intestinal flora, it also reinforces the immune defense and improves the production performances of animals (Jin et al., 2000, Coppola and Turnes 2004, Stella 2005)

*Rhodococcus+ Rhodobacter* produces L glutamic Acid and also degrades pond bottom pollution effectively.

*S boulardii* helps to improve the DO and kills many germs, worms, bacteria, fungi and virus.

*Thiobacillus ferroxidans* removes iron pyrites ; Oxidises ferrous ions, Sulphur and sulphides.

*Thiobacilus thioxidans* Controls vibrio

*Thiobacillus novellus*

**SALIENT FEATURES OF PROWASO\_VA**

* Binds Heavy Metals
* Binds and degrades Toxins
* Controls *Vibrio*
* Decreases the aeration cost in the intensive and hyper-intensive ponds through microbial action and more efficient degradation of organic material, thereby reducing bottom sedimentation.
* Decreases the pumping cost in the intensive ponds by improving water quality and decreasing the frequency and quantum of water exchange as well as black sludge after harvest.
* Degrades the pollutants at the pond bottom
* Eliminates water fouling products like Ammonia, Nitrites, Hydrogen Sulfide, Iron
* Improve feed conversion
* Improve survival rate
* Improves D O
* Improves Disease Resistance
* Increase in average weight gain.
* Increases the growth rate
* Increases shrimp quality
* Minimizes pre and post moulting mortality.
* Maintains Optimum pH
* Prevents gill rot, tail rot, antenna rot and loose shell.
* Provides ambient atmosphere to the inhabitant
* Reduce the duration of the cycle to achieve target weight.
* Reduces need of frequent water exchanges
* Reduces Turbidity
* Relieves from the stress
* Traps Virus

**Why should one use soil and water probiotics in aqua culture**

Shrimp **aquaculture** production in much of the world is depressed by disease, particularly caused by luminous *Vibrio* and/or viruses. Antibiotics, which have been used in large quantities, are in many cases ineffective, or result in increases in virulence of pathogens and, furthermore, are cause for concern in promoting transfer of antibiotic resistance to human pathogens. Probiotic technology provides a solution to these problems.

The microbial species composition in hatchery tanks or large **aquaculture** ponds can be changed by adding selected bacterial species to displace deleterious normal bacteria. Virulence of luminous *Vibrio* species can be controlled in this manner. Abundance of luminous *Vibrio* strains decreased in ponds and tanks where specially selected, probiotic strains of *Bacillus* species were added. A farm on Negros, in the Philippines, which had been devastated by luminous *Vibrio* disease while using heavy doses of antibiotics in feed, achieved survival of 80-100% of shrimp in all ponds treated with **probiotics**.

**Why only this solution better**

The solution lies in the field of microbial ecology, not in the field of pharmacology, i.e.

in developing new antibiotics or vaccines. Shrimp farmers have to learn to live with a

complex community of microbes and manage them. The use of beneficial bacteria

(**probiotics**) to displace pathogenic bacteria by competitive processes is a better remedy than administering antibiotics.

The microbial species composition in **aquaculture** ponds can be changed by adding

selected species to displace deleterious common bacteria. Success depends upon defining the ecological process or processes to be changed, the types of deleterious species that are dominant and the desirable alternative species or strains of bacteria that could be added.

Competitive exclusion is one of the ecological processes that allows manipulation of the

bacterial species composition in the **water**, sediment and animal guts.

**Why Chlorine is to be discarded**

Chlorine is widely used in hatcheries and ponds, but its use stimulates the development

of multiple antibiotic resistance genes in bacteria [8]. Some farmers in Thailand have

reported that when chlorine is used in ponds to kill zooplankton before stocking shrimp,

there is a rapid increase in *Vibrio harveyi* numbers after the chlorine is removed. This is to be expected as marine vibrios have very fast growth rates, and the chlorine treatment will lower the numbers of competitors for nutrients and kill algae, thus increasing food

resources. It is likely, therefore, that the vibrios surviving after chlorine treatment are not

only more resistant to antibiotics, but are also pathogenic.

**Why Antibiotics and disinfectants are to be discouraged**

If antibiotics or disinfectants are used to kill bacteria, some bacteria will survive, either

strains of the pathogen or others, because they carry genes for resistance. These will then grow rapidly because their competitors are removed. Any virulent pathogens that re-enter the pond or hatchery tank, perhaps from within biofilms in **water** pipes or in the guts of animals, can then exchange genes with the resistant bacteria and survive further doses of antibiotic. Thus, antibiotic-resistant strains of pathogens evolve rapidly.

The transfer of resistance to human pathogens and gut bacteria is of major concern.

Such transfers probably happen easily and often, as discussed by Salyers . Resistance

plasmids encoding for many antibiotic resistance genes were transferred between

pathogenic and non-pathogenic Gram negative bacteria in several environments including sea **water**. In the presence of tetracycline concentrations that were not high enough to kill the bacteria, the rate of gene transfer between *Vibrio cholerae* and *Aeromonas salmonicida* increased 100 times.

Throughout Asia, shrimp farmers use antibiotics in large quantities. Warehouses

supplying the industry in all the major centres sell a range of antibiotics in containers of

500 g or more in size. The antibiotics in current use include fluoroquinolones especially

norfloxacin and enrofloxacin, furazolidone, oxolinic acid, oxytetracycline, trimethoprim

and sulphadiazine. It is difficult to find out just how much antibiotic use there is in the

industry, but it is possible to make an estimate from feed usage and production. In 1994

Thailand produced about 250,000 tonnes (a quarter of the world production) of farmed

shrimps, which consumed about 500,000 - 600,000 tonnes of feed. With the disease

problems, shrimp production last year was down to 150,000 tonnes. For each crop at

semi-intensive to intensive scales of production, farmers use 5 - 10 g antibiotics per kg

feed at least once per day at weekly intervals; some use them for more extensive periods.

Thus antibiotics would be used in about 10% of feed. It is possible, therefore, that the

antibiotic usage in shrimp farm production in Thailand in 1994 was as much as 500 - 600

tonnes, assuming all farmers used them — and this does not include that used in hatcheries for fry production.

As much of this will end up producing bacteria with multiple antibiotic resistance in

farm effluents that then contaminate coastal waters, the potential impact on human health is significant. This problem was discussed by Austin in 1983 with reference to fish farming, but it has become far worse with the major increase in shrimp farming that has occurred since then.

**Why only Probiotic Bacteria is recommended?**

The term “probiotic” has been defined as: “a probiotic is a mono- or mixed culture of live

microorganisms that, applied to animal or man, affect beneficially the host by improving

the properties of the indigenous microflora” [3]. In this discussion, the authors considered only human and land farm animals. In extending their definition to **aquaculture**, I suggest that it also applies to the addition of live, naturally-occurring bacteria to tanks and ponds in which the animals live, because these bacteria modify the bacterial composition of the **water** and sediment. The health of animals is thus improved by the removal, or decrease in population density, of pathogens and by improving **water** quality through the more rapid degradation of waste organic matter.

Unlike land animals, aquatic farmed animals are surrounded by a milieu that supports

opportunistic pathogens independently of the host animal, and so the pathogens can reach high abundance around the animal. *Vibrio* grow attached to algae, and may reach high population densities after being ingested with the algae and then excreted with lysed algae in faecal pellets by zooplankton; they are gut bacteria in fish and shrimps as well as zooplankton [7]. In **aquaculture** ponds, where animal and algal population densities are very high, *Vibrio* numbers are also high compared to the open sea. The onset of shrimp disease due to exposure to high numbers of *Vibrio*, especially when pathogenicity has increased by overuse of antimicrobial compounds indicates that a defense is needed.

The species composition of a microbial community, such as that in a pond, will be

determined partly by stochastic phenomena, that is, chance, and partly by deterministic and predictable factors that allow one species to grow and divide more rapidly than others, and thus dominate numerically. Chance favours those organisms that happen to be in the right place at the right time to respond to a sudden increase in nutrients, e.g. from the lysis of algal cells or the decomposition of feed pellets that fall around them. The farmer can manipulate the species composition by seeding large numbers of desirable strains of bacteria or algae; in other words, by giving chance a helping hand.

Competitive exclusion is one of the ecological processes that can be manipulated to

modify the species composition of a **soil** or **water** body or other microbial environment.

Small changes in factors that affect growth or mortality rates will lead to changes in

species dominance. We are still a long way from knowing all the factors that control

growth rates of particular species. The complete species composition in natural

environments is largely unknown, but enough is known to argue that it is possible to

change species composition by making use of competitive exclusion principles [11]. Thus

bacteria can compete by secreting antimicrobial compounds that do not necessarily kill all their competitors, but increase mortality rates just enough to tip the balance in resource utilization.

**Is this product embedded with the latest technology**

Microbial ecology and biotechnologies have advanced in the last decade, to the point

that commercial products and technologies are available for treating large areas of **water**

and land to enhance population densities of particular microbial species or biochemical

activities. The practice of bioremediation (or bioaugmentation) is applied in many areas,

but success varies greatly, depending on the nature of the products used and the technical

information available to the end user. The bacteria that are added must be selected for

specific functions that are amenable to bioremediation, and be added at a high enough

population density, and under the right environmental conditions, to achieve the desired

outcomes. Bioaugmentation and the use of **probiotics** are significant management tools for **aquaculture**, but their efficacy depends on understanding the nature of competition between particular species or strains of bacteria. They rely on the same concepts that are used successfully for **soil** bioremediation and probiotic usage in the animal industry.

**Probiotics** such as the Gram positive *Bacillus* offer an alternative to antibiotic therapy

for sustainable **aquaculture**. *Bacillus* species are commonly found in marine sediments and therefore are naturally ingested by animals such as shrimps that feed in or on the sediment.

An advantage of using *Bacillus* species is that they are unlikely to use genes for antibiotic resistance or virulence from the vibrios or related Gram negative bacteria.

What is the shelf life of the formulation?

Stable for about Nine months from date of mfg

**What is the recommended level of inclusion?**

In pond water medium @ 1 Kg/ Acre once in 15-21 days

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