Development Plan for Aquaculture Using Photosynthetic Bacteria as Food Additives and Water Quality Improvement Agents

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ABSTRACT

This paper presents a case study to evaluate the feasibility of a development plan for aquaculture based on the application of photosynthetic bacteria as food additives and water quality improvement agents. Our take on this aspect considers the development specifically in La Union Province, El Salvador. This is due to depletion of natural resources, no recycling methods, use of harmful chemicals and the lack of advancements in the given industry. Photosynthetic bacteria improve the culture of species, recycle resources, improve system’s immunity to diseases, increases growth of species, and don’t exhaust pond’s oxygen. Results have shown that photosynthetic bacteria are the optimal solution for a safe transition from wild fishing to aquaculture. This would also be the first time ever to implement this type of technology in El Salvador.

Keywords: Aquaculture, photosynthetic bacteria, development plan, El Salvador.
INTRODUCTION

1.1 Motivation

According to recent studies done by several local organizations, La Unión province, located in southeastern El Salvador, has a very strong potential that has not yet been exploited, that is, its aquaculture industry. However, not taking advantage of this opportunity causes a deplorable quality of life for the thousands living in La Union. When bans are imposed by the Ministry of Agriculture, fishermen are forced to seek other job opportunities, which are scarce. Often the case, people commit crimes in order to get any form of income, causing crime rates to increase everyday for thousands of these families depend exclusively on fishery. It creates economic instability in the area, thus increasing crime and frightening away local and foreign investment. Tourism is null in these areas due to the lack of interest from the government. However, it is time to acknowledge the natural beauty and commerce potential of this region.

La Unión is stuck in a downturn. The main problem being that the majority of its population is dedicated to artisanal and industrial fishery, which harms the ecosystem by depleting natural resources, causing long and harsh government imposed bans. People don’t have much of a choice in this case due to lack of knowledge. The support needed to develop other activities in the region could significantly improve the quality of life for thousands of families, ultimately, the country. The few who know and use aquaculture, use it as an alter production mechanism with harmful, inefficient, and very outdated techniques.

We, as young professionals, believe in the need to develop an efficient program that allows the diversification of activities; a program that will keep a balance between the industrial-scale fishery and farming on land and sea. La Unión has a series of resources which make it suitable for the cultivation of shrimp, fish, oysters, clams, and many other species. Its geographical location (sharing territorial waters with Honduras and Nicaragua) gives it an advantage to profit not only one country, but three, and has an internationally certified new port capable of double the capacity of the currently busiest port in the country. Also, it contains more than 40 Km. of beautiful beaches that lack of tourism infrastructure that would make it the ideal place to invest.

Creating a development plan along with teaching the population about aquaculture and its benefits could change the way people think in this area. New techniques, such as the use of Photosynthetic bacteria and other probiotics, have a more digestible bacterial cell wall, and are rich in proteins, carotenoids, biological cofactors, and vitamins, for their various metabolic pathways for the degradation of organic wastes can be a strong alternative (Kobayashi and Kurata, 1978). Organic enrichment and nitrogenous waste, including ammonium and ammonia are a serious concern in aquaculture, thus we can use bacteria for improving water quality. It was reported that the addition of photosynthetic bacteria as food additives stimulated the growth of shrimp and fish (Zhang et al., 1988), enhanced the survival rate of fish larvae, and improved the production of scallop seed (Huang et al., 1990; Wang et al., 1994). This could minimize the use of antibiotics, which harm the environment and strengthen virus.
1.2 Case Study

Nowadays, aquaculture is the fastest growing food-producing sector in the world, with an average annual growth with a rate of 8.9% since 1970, compared to the only 1.2% for capture fisheries. World aquaculture has grown significantly from less than a million ton to 60 million tons by 2004. The level of production had a value of US$70.3 Billion that same year. Of this production, China produced the 70% and the rest were produced in other parts of Asia and Pacific region.

Sadly, this sector has just begun to develop in El Salvador, where the lack of awareness has caused the country to be currently facing the dangers of depleting the majority of its marine resources. The country needs a significant shift from wild fishing to aquaculture in order to prevent depleting our resources and to improve the aquaculture industry. Such a shift would seem rather radical, but research proof otherwise, for the same case took place in China during the 1980s (S.F. Li, 2000). As a result of this significant shift, aquaculture development in China has accelerated throughout the years and spread throughout the country, making it the world’s largest producer of aquatic products today.

Due to the lack of new technologies, El Salvador’s aquaculture sector still faces a lot of problems, the biggest being diseases. These diseases are usually controlled by harmful chemicals, which not only put the pond’s health at stake, but can also harm the animals and pollute the water beyond repair. Diseases are listed as the primary constraint to a sustainable development of the aquaculture industry, which impedes both economic and social development in many sectors of El Salvador.

In the following sections we will review how to create a development plan based on the use of photosynthetic bacteria as a new technique to culture aquatic species, the economic values and social benefits of such technique, followed by the development of proper infrastructure that will allow us to implement such projects in the southeastern coast of El Salvador. We will also address the solutions to help change the mentality of habitants who strictly depend on wild and industrial fishery. This will be achieved by comparing and analyzing the actual techniques used in El Salvador to culture aquatic species with the ones used abroad, as well as the harm that this outdated techniques are causing to the environment. For further awareness, this rely on visual aid such as info graphs or billboards.

Through research, it has been discovered that Probiotics inhabit the growth and reduce pathogenic bacteria, at the same time they enhance the nutrition of the cultured species, improve the quality of water thus reducing the use of antibiotics and chemicals that harm our ecosystems. In the early 90's some hatcheries in Taiwan and Thailand were promoting the use of probiotics, but these ideas were not readily accepted by the more scientific operations (Gomes, 1992). Today the concept of probiotics is reviewed by leading scientists (Verschuere et al, 2000). Verschuere et al defines aquaculture probiotics as "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment."
2.1 The Photosynthetic Bacteria

Our goal is to develop microbial-controlled ponds, which will give us a stable control over processes in the ponds. In order to achieve this, we need to increase the degradation of organic matter, enable efficient nitrification, and the recycling of nitrogen. Experience acquired by these countries gives us the chance to use well developed techniques without struggling to succeed in their use. Our task is simply adapting all the methods compiled to our environment. We need to realize the great economic values and the potential social benefits of the application of Probiotics in aquaculture and pay more attention to the development of techniques that will give us the best qualities of Photosynthetic bacteria, thus better results in production.

In English literature, probiotic bacteria are generally called the bacteria which can improve the water quality of aquaculture, and (or) inhibit the pathogens in water thereby increasing production. "Probiotics" or "beneficial bacteria" are the terms synonymously used for probiotic bacteria. The term Probiotic (the opposite of the term antibiotic), meaning “for life” in the original Greek Language, was previously defined by Fuller (1989) as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.” The development of non-antibiotic and environmental friendly agents is one of the key factors for health management in aquaculture. In Aquatic species such as fish and shrimp, the occupation of the gastrointestinal tract begins right after hatching and is completed within a few hours to modulate expression of genes in the digestive tract, thus creating a favorable habitat for them and preventing invasion by other bacteria introduced later into the ecosystem (Balcázar et al., 2006)

Application of probiotics as we know it nowadays began to be applied in Chinese aquaculture since the 1980s. For more than a decade, there has been an exponentially growing application of probiotics in aquaculture: at present more than hundred companies are producing many types of probiotics for aquaculture, and probably over 50,000 tons of commercial probiotic products are sold annually with a market value estimated at $60 million US. The probiotics used in Asian aquaculture are mainly photosynthetic bacteria (PSB), antagonistic bacteria (Pseudoalteromonas sp., Flavobacterium sp., Alteromonassp., Phaeobacter sp., Bacillus sp., etc.), microorganisms for nutritional and enzymatic contribution to the digestion (lactic acid bacteria, yeast,etc.), bacteria for improving water quality (nitrifying bacteria, denitrifiers,etc.), Bdellovibrio, and other probiotics (Zizhong Qi a, Xiao-Hua Zhang a, Nico Boon b, Peter Bossier). All of the previous are the optimal mix to carry out a successful system.

Currently, photosynthetic bacteria are found in five bacterial phyla, i.e. Chlorobi, Cyanobacteria, Chloroflexi (filamentous anoxygenic phototrophs), Firmicutes (heliobacteria) and Proteobacteria (purple sulfur and purple non-sulfur bacteria) (Bryant and Frigaard, 2006).

Purple non-sulfur bacteria are widely distributed in freshwater, marine, soil and hot-spring environments, which can be favorable for El Salvador, due to its tropical weather. They have various metabolic pathways for the degradation of organic wastes, which is one of the key
points for a clean and stable pond. They also have a more digestible bacterial cell wall, and are rich in proteins, carotenoids, biological cofactors, and vitamins (Kobayashi and Kurata, 1978).

The species currently used in Chinese aquaculture are Rhodopseudomonas palustris, Rubrivivax gelatinosa, Rhodobacter capsulata, R. sphaeroides, Phaeospirillum fulvum, etc. Those are probably the earliest and the most widely used probiotics in China since the 1980s. It was reported that the addition of photosynthetic bacteria as food additives stimulated the growth of shrimp and fish (Zhang et al., 1988), enhanced the survival rate of fish larvae, and improved the production of scallop seed (Huang et al., 1990; Wang et al., 1994). They were also found to increase the population growth rate of live food such as Brachionus plicatilis (Xu et al., 1992). Furthermore, some plant and herb extracts were identified as growth promoter for the mass cultivation of photosynthetic bacteria (Xu et al., 1994). The technique which uses photosynthetic bacteria as probiotics has become common practice in China. Due to the convenience of these products, Asian farmers today are using concentrated and encapsulated commercial photosynthetic bacterial products, simple as that.

Since El Salvador lacks of experts in the matter, the most viable way is to buy such products from China while experts are forged in this field and can produce them. Many commercial photosynthetic bacterial products are labeled as either single or multiple species at concentrations higher than 109 ml⁻¹, depends on what is wanted to achieve, and are often combined with growth promoters or conditioners, and are claimed to have multifunctional effects such as improvement of water quality, enhancement of growth rate and prevention of disease (Zizhong Qi, Xiao-Hua Zhang, Nico Boon, Peter Bossier).

Maeda and Nagami (1989) reported some aspects of the bio-controlling method in aquaculture. In their study bacterial strains possessing vibrio (waterborne bacteria) stationary activity which improved the growth of prawn and crab larvae were observed. By applying these bacteria in aquaculture, a biological equilibrium between competing beneficial and deleterious microorganisms was produced, and results show that the population of Vibrio spp., which frequently causes large scale damage to the larval production, was decreased. Survival rate of the crustacean larvae in these experiments showed much higher than those without the addition of bacterial strains. They hope that addition of these strains of bacteria will repress the growth of Vibrio spp., fungi and other pathogenic microorganisms. Their data suggest that controlling the aquaculture ecosystem using bacteria and protozoa is quite possible and if this system is adopted, it will maintain the aquaculture environment in better condition, which will increase the production of fish and crustaceans.

2.2 Characteristics of Photosynthetic Bacteria (P.S.B.):

- P.S.B. is used for maintaining the quality of water and bottom pond soil.
- P.S.B. is poisonless and requires very little oxygen. Thus, P.S.B. will not exhaust the oxygen in the pond.
- Posses an excellent capacity of purifying water quality. It is so called scavenger of pond.

2.3 Functions:

- Improve the water quality of pond.
• Contain special active bacteria which can decompose the organic materials, NO₂ and harmful materials of pond.
• If raining, the pond may be easily polluted by acid rain. P.S.B. can purify the water quality and increase capacity of oxygen dissolution.
• Increase the capacity of disease resistance:
  • Prevent fishes and shrimps from disease and ensure their health.
  • Reduce the use of antibiotics and chemicals.
• Rich in nutrition.
• Contains plentiful protein, vitamin B and so on. Add in feedstuff can supply additive nutrition which is lacked in normal feedstuff.
• Contain large amount of carotene and vitamin A and D. P.S.B. can enhance the body color of fishes and shrimps.
• Improve the appetite and digestion of fishes and shrimps.
• Promote the growth of botanical plankton (algae).

MATERIALS AND METHODS

3.1 Experiment

This experiment was produced in order to prove that the bacteria can be cultured in a small lab without difficulty.

Steps:

1. Poured the water into the saucepan until it boiled.

2. Added Agar powder to the boiling water and stirred for a minute until the entire ingredient dissolved. (Fig.1)

3. Cooled agar mixture slightly for 10 minutes. The mixture needs to be still hot to avoid the gelatin setting in the saucepan and to prevent contamination from bacteria in the air. The conditions are far from sterile; we wanted to avoid as much contamination as possible.

4. Took the lid off the Petri dishes and filled the Petri dish with the hot mixture. We only took the lid off the Petri dish when we were ready to pour the agar, or they would have become contaminated with the bacteria in the air.

5. Immediately put the lid back on the Petri dish and put it in the fridge for about 4 hours until the agar has set. Do not touch the agar or you will contaminate it with bacteria on your fingers.

6. Now it’s time to collect and grow your bacteria (or fungi) on the agar Petri dishes. Note: the Petri dishes can be stored in the fridge for 1-2 days before use.
7. The Bacillus photosynthetic bacteria were provided by our professor, and acclimated to room temperature under shade conditions in a 3000 ml plastic container. All trials for photosynthesis with respect to depth, trials for mortality and exploitation rate were performed at Prof. Chen’s Research Lab (Kun Shan University, Taiwan).

8. We collected soil from three different depths in order to obtain Bacillus bacteria and other bacteria that work along with this one. After collected, rub each sample lightly across the agar in a zig-zag pattern.

9. We put the lids back on the Petri dishes, labeled them, taped them closed and placed them upside down at room temperature for 3-5 days, where they received sunlight.

10. After waiting for 3-5 days, we did all the proper steps to observe them in the Microscope. We successfully cultured bacteria using home tools. (Fig.2)

3.2 Sampling

Photosynthetic bacteria (*bacillus*) were separated with different concentration of nutrients and levels of soil and water obtained from a local supplier.

3.3 Materials

- ½ teaspoon of agar powder
- ¼ cup of water
- Saucepan for boiling mixture
- 3 x Petri dishes
- Spoon
- Sticky tape
- Felt-tip pen to label petri dishes
- Cotton swab (optional)
- 35°C to 37°C incubator (optional) – oven, warm spot behind fridge, near a heater, box with a desk lamp inside or on top.
3.4 Mixing with harvest species

This experiment consisted of culturing bacteria out of 3 samples given to us. The samples cultured were obtained from our advisor. They had been previously used to culture aquatic species. After performing the experiment successfully we introduced bacteria to a pond full of harvest species, which had never been in contact with Probiotics. We obtained positive data on how the bacteria affect the ecosystem and the species inside it. Details are explained throughout the paper.

![Fig.3 Samples.](image)

3.5 Current Solutions

**Liable Suppliers due to the lack of Probiotics Producers in El Salvador.**

We have identified pioneer companies which produced the earliest and some of the currently used probiotics. Nowadays, the most popular one in China is Effective Microorganisms (EM) from Dr. Teruo Higa's EM Technology, Japan. The original developer of the technology that combines microorganisms for various beneficial uses, the Japanese agronomist Higa named his discovery “Effective Microorganisms™” or “EM” and started to use the term internationally in 1986.

Alken Murray has taken this definition a bit further, in that specific probiotic blends are designed to handle specific functions, by producing a dry, synergistic blend of fourteen spore-forming *Bacillus* strains and eight gram-negative vegetative strains of bacteria called Alken Clear-flo 1005 for sludge decomposition in aquaculture ponds. Another probiotic, *ALKEN CLEAR-FLO 1006*, is a blend of natural bacteria specifically designed to discourage disease proliferation in aquatic environments by enhancing immune response of cultured species while eliminating specific pollutants that foster pathogenic *Vibrio spp.* and other disease causing species. This formula uses six gram-positive *Bacillus* and ten gram-negative vegetative strains that aggressively consume a broad spectrum of pollutants, including surfactants, fats, sugars, starch, and nominal amounts of pesticides and hydrocarbons, while eliminating the formation of hydrogen sulphide (Laurence Evans-Mtizuni, Prawns of South Africa).
DISCUSSION

4.1 Comparison of the techniques used in El Salvador and Central America

In El Salvador, the culture of fresh water prawn is in its earliest stage; in 2009 CENDEPESCA identified a total of 25 Aquaculture Farms. Out of 25 farms, 12 culture sea water shrimp (Litopenaeus vannamei) and the rest fresh water prawn (Macrobrachium rosembergii) (Lin Ruey-Chyi, Desarrollo larvario de camarón de agua dulce Macrobrachium rosembergii).

The cultivation of marine shrimp (Litopenaeus vannamei and L. stylirostris) in El Salvador is concentrated mainly in the eastern margin of the Bajo Lempa and Jiquilisco Bay (Fig.4), estimated in 1995 that between 580 and 780 hectares (Currie, 1995a). Most of that extension (»97%) corresponds to salt evaporation ponds converted to shrimp farms. They led to the reintegration of ex-combatants in the civil war. (Rauda R. Hernandez 1, 2 and W. López Martínez Vásquez M. Jandres 1). We want to achieve similar results, but this time including a sector of the place that has been margined.

The origin of the freshwater prawn (M. Rosembergii) places it in Asia, developed in tropical climate, it’s the largest species in the world and quickly adapts to the environment. It is omnivorous; its growth is fast and easy to be cultured. In El Salvador, the climatic conditions are favorable for cultivation throughout the year, supported by the quality and availability of fresh water, tracts of land suitable for cultivation and availability of labor force. Whereas shrimp post-larvae can’t be gathered from the natural environment, they need to be developed in an appropriate laboratory and controlled to be able to meet the demand for post-larvae to farmers interested in culturing (Lin Ruey-Chyi, Desarrollo larvario de camarón de agua dulce Macrobrachium rosembergii). Nowadays, there are only 5 laboratories available and 3 of them produce sea water shrimp post-larvae, the rest produce fresh water prawn larvae.

Fig. 4
4.2 Types of Techniques used to culture aquatic species in El Salvador

1. Craft (Artisanal) System

Very short production cycles (6-8 a year) or long (one year), last 45 or 60 days and the second from 180 to 240 days characterize this technique. The post-larvae supply is strictly wild by opening gates without selection at high tide and the water supply is by opening gates in the days preceding and following phases of full and new moon, when tides are higher (> 2.9 meters). The yield is lower than 720 lbs / hec / year.

2. Extensive Enhanced

Units operating under this concept also carry out three production cycles per year (> 90 days) and are supplied with water by opening floodgates on days with higher tides. It differs from the old system (uses only wild post-larvae) because it avoids the input of wild post-larvae and other undesirable species placing frames serially from 4 x 4 (6.4 mm^2) to 64 x 64 (0.4 mm^2) and exchanging when clogged with trash. The ponds are filled with post-larva single laboratory, at a density of 5-8 per square meter, depending on the facility to refill the water. Water exchange rates are restricted to days with higher tidal height (> 2.9 m), in line with the 3 or 4 hours actual tide. All other cultural practices are similar to the extensive system. The yield varies between 1,000 and 2,200 pounds / hec / year.

3. Semi-Intensive System

It differs from the previous one by the following characteristics: production units are supplied by diesel-driven pump, usually from 30.5 to 40.6 cm. flow. This facility allows stock up on more days of the lunar month to not rely so heavily on higher tides. If the ponds have channel reservoir, the water changes are more frequent ensuring better quality growing environment. The below facilities (fig 2,3,4,5) allow exclusively populate ponds laboratory seed densities between 10 and 20 post-larvae per squared meter. All other cultural practices are similar to the previous system. Usually, the performance is superior to 2.200 lbs / hec / year.

The problem is that these techniques cause the depletion if wild larvae, which harms the production of shrimp. It shows high rates of mortality when captured. There’s also no control of virus entrance into the pond. Our solution is to use Probiotics in order to put an end to these constraints.
4.3 Place to Develop the Plan

- **La Unión Province**
  - Is a municipality in the department of La Unión (Fig.5,6,8,9) of El Salvador. It is the capital city of the Department of its same name. It’s the largest city in the department with a population of 26,739 inhabitants. Most of them depend on fishery as a job and lifestyle. On January 16, 2005, the government of El Salvador started building a new port (Fig.7), in order to replace the old one. It was finished two years ago, currently looking for a multinational enterprise who can be able to run it. It will be able to hold more than double the cargo held by the current principal port of El Salvador at Acajutla.

**Total extension:** 144.43 km² (55.76 sq mi).

**Elevation:** 5 m (16 ft).

**Population (2012) Total:** 29,739.

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**Fig.5 Department of La Union, El Salvador.**

**Fig.6 Satellite image of La Unión**

**Fig.7 La Unión Port**
4.4 Raw materials availability

It is very important to administer larvae in a proper manner, whether they are to be cultured through wild fishing or in a lab. Larvae must have good development from the beginning in order to have good results in the future. La Union province is provided with both seawater and engulfed water from el Golfo de Fonseca (Fig.9), which makes it most favorable for culturing and establishing of labs that could supply both larvae and probiotics to the region, which could include neighboring countries such as Honduras and Nicaragua, making the project have a wider reach in terms of development. This could account for the distribution of raw material and future cooperation between nations in research & development.

Shrimp culture, along with species’ culture, represents one of the 4 major products of El Salvador economically speaking. Ever since 2000, fishery has decrease drastically due to the depletion of natural resources and overfishing. (Indicadores macroeconómicos del sector pesquero y acuícola del Istmo Centroamericano 2000-2007)
CONCLUSION

We concluded that the ecosystem of an arable farm can be altered positively by introducing probiotic bacteria which, when mixed with the environment and with the crop species provide a balance that stops the development of virus and diseases, giving better results and an expected bumper harvest. Achieving the development of an industry that would benefit the country both economically and socially is our goal. On the long run, we also aspire for this project to become a reality in Honduras and Nicaragua as well. Abundant land and labor is available, all we need is technical support to carry it out.

Through research we have managed to ensure that these techniques are viable and can be applied both in El Salvador and neighboring countries. It is important to remember that these techniques will help us to achieve a transition from industrial-scale fishing to aquaculture practice on a larger scale in this area of the country. We want to develop this project and urge the government to launch the first fish farm development program in the country. Only this time, we will use cutting-edge techniques that enable us to compete with developed countries that have today’s market share in aquaculture.
The following are images depicting shrimp farms in Usulutan Province, El Salvador:
OTHER REFERENCES


Chart Chiemchaisri, Lumpoon Jaitrong, Ryo Honda, Kensuke Fukushi, and Kazuo Yamamoto
(Kobayashi and Tchan, 1973; Getha et al., 1998; Banjaree et al., 2000)