



Ecotech: A Probiotic for maintaining water quality and Control of *vibrio sp in vitro*

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Received 18 October, 2015; Accepted 20 November, 2015 © The author(s) 2015. Published with open access at www.questjournals.org

ABSTRACT:- In aquaculture practices, probiotics are considered a valid alternative to antibiotics and in particular, to maintain water quality and to control pathogen loads. In the *In vitro* study; the concentrations of ammonia, nitrate and nitrite were increased artificially by treating water with nutrients. The Ecotech (Microl Remedies product) as a probiotic blend was added to the treated water 4 mg to 5 mg in 50 L. (the dosage sets as per field condition). A Negative control group (without Ecotech) was also studied for comparison. All trials were replicated. Ecotech lowers the ammonium, nitrate and nitrite levels in water after 12hr of treatment. *In vitro* antagonism test of the Ecotech against shrimp pathogen *Vibrio sp.* was performed by using well diffusion method, effective zone of inhibition of 2.4 cm was observed. The results demonstrated that Ecotech could be used as probiotic against pathogenic *Vibrio sp* in shrimp industry.

Keywords:- Probiotic, Well Diffusion, *Vibrio sp*, Biocontrol

I. INTRODUCTION

Increase in aquaculture has been accompanied by outbreaks of disease from an ever-increasing range of Aquaculture is the fastest growing food-producing sector in the world, with an average annual growth rate of 8.9% and practiced in a variety of agro-climatic zones ranging from tropical to temperate area (Subasinghe, 2005). The production in aquaculture is hampered by disease caused by various fish pathogens and is constraint to the culture of many aquatic species (Bondad-Reantaso, 2005). The persistent disease problems in aquaculture necessitate the use of bacterial control agents as probiotics which can be effectively used as an alternative to antibiotics.

Lactic acid bacteria have been used as probiotics against shrimp pathogens (Gatesoupe 1999; Skjermo and Vadstein 1999) and it was used as an alternative to antibiotics in disease control strategy (Fuller, Turvey 1971, Parker 1974, Roach S and Tannock 1980, Fuller 1978, Smoragiewicz *et al.*, 1993). A sterile pond may increase disease risk substantially. Since any microbes that enter the system might easily take over. To reduce the risk, experiment is made to introduce probiotics that is “friendly microbes” in the farming environment to suppress and out compete pathogenic ones (Moriarty, D.J.W., 1998). The common probiotics used in aquaculture belonging to genus *Lactobacillus* (Rollo *et al.*, 2006), *Bacillus sp.*(Banerjee *et al.*, 2007), *Bifidobacterium sp.*, *Vibrio sp.* (Li *et al.*, 2008), *Saccharomyces sp.* (Ahilan *et al.*, 2004; Aubin *et al.*, 2005; Fazeli and Takami, 2006), *Enterococcus sp.*, *Bacillus subtilis* (Ghosh *et al.*, 2008), are now used for oral bacteriotherapy in aquaculture. Hence the present work carried out to investigate the efficacy of Ecotech on water quality and pathogen control.

II. MATERIALS AND METHODS

Bacterial culture

The common shrimp pathogenic bacteria *vibrio sp.* was isolated from the shrimp infected pond, Bhimavaram, Andhrapradesh (India).

Preparation of water

Preparation of water: 50 L of Normal water was distributed into aquarium and maintained each treatment in duplicates. Water ppm levels are artificially increased by adding nutrients ammonium chloride, calcium nitrite and calcium nitrate to it. Ammonium level (1.75 ppm), Nitrate level (0.05 ppm) and nitrite level (0.5 ppm) are maintained. Negative control is without product.

Ecotech (Probiotic blend)

The Ecotech (Probiotic blend) – 500g/Acre was used for to maintain water quality and inhibit the pathogen control; Ecotech composition: *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megatherium*, *Bacillus polymyxa*, *Bacillus pumilus*, *Lactobacillus plantarum*, *Pediococcus acidilacti*, *Paracoccus denitrificans*, *Cellulomonas cartae*, *Paracoccus pantotrophus*, *Thiobacillus sp.*, *Saccharomyces cerevisiae*, *Nitrosomonas sp.*, *Nitrobacter sp.*, *Rhodococcus sp.*, *Rhodobacter sp.*, *Aspergillus niger*.

Dosage – 500 g/Acre @5 ft or 4 ft of water

50L. water of aquarium corresponding 4.5 ft of water

As reference value dosage of Ecotech is 4 mg to 5 mg/50 L. aquarium

In vitro zone of inhibition test

Vibrio sp bacterial cultures were spread on nutrient agar plates and these plates incubated at $32\pm 1^{\circ}\text{C}$ for 24 hrs. Three to four colonies were selected and transferred into 5ml nutrient broth medium and further incubated at $32\pm 1^{\circ}\text{C}$ for 6-8 h. Sterile cotton swab was dipped into the bacterial suspension and pressed along the walls of tubes to remove excess of culture The entire agar surfaces were streaked with the swab. The inoculum was allowed to dry for 10-15 min with closed lid. The discs were dipped in Ecotech probiotic blend solution, minimum effective dose (approx $10\mu\text{l}$) and placed inside culture plates under aseptic conditions and incubated at $32\pm 1^{\circ}\text{C}$ for 24 h. After incubation the plates were observed and the diameter of inhibition zone was measured.

III. RESULTS

Water Quality Analysis:

The results of ammonia concentration showed that the values recorded were much lower in both probiotic treated aquarium than that of control aquarium. Concentrations of nitrate and nitrite were higher in control aquarium than the Ecotech treated aquarium (Table 01)

Table 01: Nutrients (ppm) notices in the present study

Particulars	Control (ppm)	Ecotech (ppm)
Ammonia-NH4	1.75±0.1	0.21±0.01
Nitrate-No2	0.051±0.05	0.0032±0.001
Nitrite-NO3	0.5±0.03	0.013±0.002

Antagonistic test:

The maximum values of zone of inhibition against the shrimp bacterial pathogens were given by Ecotech. The maximum zone of inhibition observed for Ecotech was 2.4 cm against *Vibrio sp.*(fig.1b & 1c). Control is without product and no zone of inhibition observed (fig.1a). The overall statistical significance among the replicates was negligible due to less variation in the duplicate values. The statistical significance was very less. The values of critical difference and critical variance were 2.4 and 2.38, respectively.

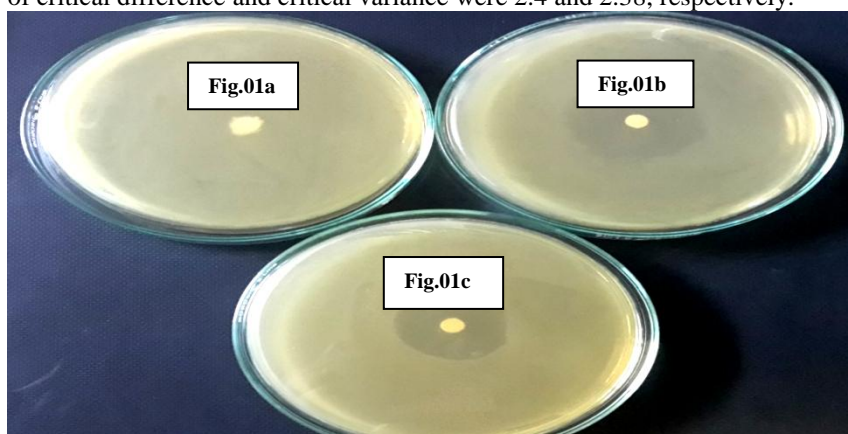


Figure 01 - Growth inhibition by Ecotech against shrimp bacterial pathogens

IV. DISCUSSION

Ammonia is the principal end product of protein catabolism of organisms and it is excreted through gills. It is also formed by decay of organic matter. When the ammonia concentration in the culture medium increases, excretion of ammonia by cultured organisms decreases. Under farm conditions, the ammonia level should be less than 1 ppm. In the present study, the level of ammonia was decreased, this is mainly due to microorganisms (*Nitrosomonas*) present in the Ecotech which initiate nitrification. Due to this process ammonia is converted into nitrite which is further acted upon by the nitrobacter and converted as nitrate through the process nitrification. Ravi *et al.* already described the benefits of probiotics in maintaining water quality and enhancing growth rate in Indian white shrimp, *P. indicus*.

The safe alternative to antibiotics i.e. commercial preparation of probiotics (Aquapro™, Exide™) and single probiotics (*L. sporogenes*, *S. boularidia*, *Rhodococcus* sp., *Nitrosomonas* sp. and *A. oryzae*) were used to study their antagonistic behaviour against the various fish pathogenic isolates. Fuller (1989) reported that probiotic preparations may be made up of a single strain or may contain mixture up to eight strains. The advantage of multiple strain preparations is that they have more sensitivity towards pathogens and active against different aquaculture animals. The probiotic preparation includes a combination of bacterial strains *viz.* *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Bifidobacterium* sp. and *Escherichia coli*. Venkatesan *et al.*, (2012) isolated probiotic organisms, *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cerevisiae* from soil, curd and yeast pellets and was that *Bifidobacterium* sp. had the high inhibitory effect against *Salmonella* sp supporting that single probiotics are also effective against bacterial pathogens. Several pond probiotics has been launched in the market search for isolation of potent probiotic bacteria with suitable pond application is a never ending process. This indicates the importance of pond environment in the animal production (Lakshmi *et al.*,) In the present study we have reported that Ecotech is mixture of micro-organisms, gave better zone of inhibition and a minimum effective dose (10µl) of Ecotech was sufficient to inhibit the growth of pathogenic *Vibrio* sp in shrimp industry.

ACKNOWLEDGMENT

We wish to express our gratitude to Dr. Veera raghavaiah and Dr. G. Rahul gaddipati for their constant support during this study . This research was funded by the MICROL REMEDIES kukatpally, Hyderabad.

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