© 2018 IJSRST | Volume 4 | Issue 5 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Applications of Bacillus sp in Aquaculture Waste Water Treatment

K. Venkateswar Reddy [1], A. Vamshi Krishna Reddy [2], B. Suresh Babu[1], T. Vijaya Lakshmi [2]

[1]Center for Biotechnology, Institute of Science and Technology, JNTUH, Kukatpally, Hyderabad-500085, Telangana. India

[2]Center for Environment, Institute of Science and Technology, JNTUH, Kukatpally, Hyderabad-500085, Telangana. India

ABSTRACT

A number of biological products including live bacterial inocula, enzyme preparations are being promoted for use in improving the water quality in aquaculture systems. In the present study, the isolated cellulolytic bacteria from polluted soil samples of pulp and paper mill effluents were developed a mixed consortium in the form of commercial probiotics. These probiotics in general are helpful in reducing the BOD, COD and nitrogenous compounds in the aquaculture ponds. Attempts have been made in the present study to use the isolates as probiotics in aquaculture ponds. The results indicate that, feed with 109 CFU g-1feed/ m3 is the best suited for reducing the BOD, COD, Phosphate and nitrogenous compounds in the aquaculture ponds. The study proved that the probiotics used was instrumental in improving the water quality in aquaculture ponds. The novel thought behind this study is that aquaculture waste water instead of discharging into the open system can be reused again after bioremediation.

Key Words: Bacillus sp., Aquaculture waste water, Physico-Chemical parameters, Applications.

I. INTRODUCTION

The term "probiotics" derived from a Greek word "pro" and "bios" meaning "prolife" (Nikaido, 2009). According to the current adopted definition by FAO/WHO, probiotics are: "Live microorganisms which are administered in required amounts confer a health benefit on the host" (FAO/WHO, 2006). Since past 50 years ago being a scientific and commercial interest in the use of beneficial bacteria for the prevention and treatment of diseases (Cutting, 2011; Gatesoupe, 1999). The first probiotics perceive long time ago was Lactobacillus sp., the lactic acid producing bacteria. There after many probiotics such as Aeromonas hydrophila, Altermonas sp, Bacillus subtilis, Lactobacillus helveticus, Lactobacillus plantarum, Micrococcus luteus, Pseudomonas fluorescens, Saccharomyces cerevisiae and Vibrio alginilyticus were considered used in aquaculture (Irianto et al., 2002) 15 species of Bacillus are the main components for the preparation of any commercial probiotics for pond aquaculture.

The mixed microbial consortia in the form of a commercial probiotics (Bacillus subtilis, Nitrosomonas, Nitrobacter and Rhodobacter) improve the water quality in aquaculture ponds (Sonia et al., 2015). Barak et al., (2003) reported that bacteria isolated from marine environments can remove specific levels of phosphorous and nitrogen. The use of Bacillus sp. improved water quality, survival and growth rates, and the general health status of juvenile penaeus monodon while also reducing pathogenic Vibrio sp. (Dalmin et al., 2001). Venkateswar (2007) reported the use of multiple strains of bacteria like Bacillus subtilis, Bacillus acidophilus, Bacillus licheniformis, Nitrobacter sp, Aerobacter and Saccharomyces cerevisiae improves the water quality and health status of fish in aquaculture. The aquaculture ponds inoculated with Bacillus sp. improves the water quality by reducing the ammonia, nitrite, phosphates, chemical oxygen demand and increased shrimp harvest (Porubcan, 1991a, 1991b). The use of probiotic microflora in aquaculture water reduces the pathogenic microorganisms to increase decomposition of harmful organic substances in the water and improves the ecological environment of aquaculture (Xiang-Hong et al., 2000).

II. MATERIALS & METHODS

Cellulolytic Bacterial Isolates:

Bacterial strains:

Bacillus subtilis sub sp. subtilis (JCEN1&JCEN2), Bacillus mojavensis (JCEN3) &Bacillus cereus (RW) strains previously isolated and identified from pulp and paper mill effluent contaminated soils were used as potential probiotics. All strains were preserved at – 20^0C in Luria broth with 15% sterile glycerol, prior to use.

Preparation of feed:

Isolated cellulolytic bacterial strains such as JCEN1, JCEN2, JCEN3 & RW were grown in LB broth using a shaking incubator at 30^0C for 48 hrs.The cultures were then centrifuged at 3000 g for 10 min at 4^0 C and, bacterial pellets were re-suspended and washed three times in Sterile Normal Saline Solution (NSS, 0.9% NaCl). The cell densities of the suspensions were measured using spectrophotometer at 600 nm, and were correlated to the colony- forming units (CFU). These suspensions were kept at 4^0C until further used. Commercial feed was used as the basal diet for the supplementation of isolated strains. All isolated cellulolytic bacterial cultures were mixed in equal proportions with the feed to give final concentrations such as 10^5 (T-1), 10^7 (T-2) & 10^9 (T-3) CFU g^{-1} of feed. The amount of the isolated strains in the feed was determined using the plate count technique. Briefly 1 g of each type of feed was randomly sampled and serially diluted (10 fold) in phosphate- buffered saline solution (PBS; pH 7.2). Then 0.1 mL of each dilution was spread on Mannitol egg Yolk Polymyxin agar (MYP agar) in order to enumerate the cell number of isolated bacteria (CFU g^{-1}) (Hadi Zokaeifar et al., 2012).

Physico - Chemical Characters of Aquaculture Waste Water Sample:

The physico chemical characteristics of the waste water viz., temperature, pH, Dissolved oxygen, BOD, COD, alkalinity, hardness and nutrients total ammonical nitrogen (TAN), nitrite-N, nitrate-N and phosphate were determined following the standard protocols from APHA (2012). These parameters were determined before and after the addition of isolates. The results of the study were analyzed statistically using ANOVA and the level of significance at $P<0.05$ & $P<0.01$ were considered for validation (Snecdecor & Cochran 1967).

Application of isolates in aquaculture ponds:

A mixed culture of bacterial strains such as JCEN1, JCEN2, JCEN3 & RW was applied to the pond separately at concentration of 10^5 (T-1), 10^7 (T-2) &10^9 (T-3) CFU g^{-1} of feed / m^3 (Table 1). Initially, mixed culture was activated by mixing it- in 500 mL of water sample and left aside with continuous aeration for 2 hours. Then the isolates were applied in ponds uniformly.

III. RESULTS & DISCUSSION

Assessment of microbial isolates in treatment of aquaculture waste waters:

The utilization of different types of antimicrobial agents as a preventive measure is in practice for disease control in aquaculture. In view of the risks associated with the use of antibiotics; the development of non pathogenic agents is one of the key factors for health management in aquaculture. According to Browdy (1998), one of the most significant technologies that evolved in response to disease control and Water quality improvements in aquaculture was the use of probiotics. The researches have been attempting to isolate beneficial bacteria from various sources like Soil, Water and animal gut to control disease causing pathogens in aquaculture systems (Bestha lakshmi et al., 2013).

In our study Cellulolytic bacterial strains were used as a potential probiotics for improving water quality in aquaculture ponds. The mixed culture of cellulolytic bacterial strains (JCEN1, JCEN2, JCEN3 & RW) at three different concentrations 10^5 (T-1), 10^7 (T-2) & 10^9 (T-3) CFU g^{-1} of feed/m^3 were tested. They effectively reduced the nitrogenous compounds, phosphate-P, BOD, and COD from the aquaculture ponds.

The raw aquaculture waste water quality parameters were determined before and after bioremediation in T-1, T-2 and T-3 treatments, and results were represented in table 2.0 The pond water temperature was found to be in between 28-30^{0}C. The pH of treated pond water was slightly less than the control during bioremediation and this might have been due to enhanced microbial activity leading to greater level of respiration and increase in carbon-di-oxide. The fluctuation of temperature is not only increasing bacterial pathogens but also will influence viral virulence for mortality in the aquaculture species (Albert & Ransagan 2013).Temperature is known to regulate the growth and other physiological and biochemical functions of fish. The combinations of probiotics (Bacillus subtilis, Lactobacillus lactis, Saccharomyces cerevisiae) were used to overcome the temperature associated stress, which has also substantially improved the fish growth(Mohapatra et al., 2014).The initial TAN value of raw aquaculture waste water was 0.916 mg/L higher than the permissible limit of 0.1 mg/L (Boyd and Gross, 1998). The level of TAN cannot be removed easily however it can be converted to non toxic nitrate by nitrifying bacteria. The rate of removal of TAN was higher in T-3 when compared to other two treatments and control. At the end of bioremediation, there was 91% removal of TAN in T-3. Bacillus sp would have improved the nitrification process and converts the TAN into Nitrite-N and Nitrate-N. Nitrate concentration in T-3 was more; it might be due to oxidation of various forms of inorganic nitrogen. A favorable range of Nitrate 0.1 to 4.0 mg/L as admissible was reported by Sahar (2014). The values of nitrate-N were within the safe limit. Bacillus sp can utilize ammonia via both heterotrophic and chemotropic pathways (Edwards, 2011).This may be the reason for enhanced removal of ammonical nitrogen from waste water only after the addition of the cultures of Bacillus sp (Sharma et al., 2014).Some of the biological processes like aerobic followed by anaerobic treatment involving bioaugmentation using the culture of Bacillus sp seems to be a good and economic solution for treating the fish processing industry waste water with high ammonical nitrogen (Sarnaik et al., 2015).The Dissolved oxygen concentration is an index of water quality. The Dissolved oxygen content increased from 1.08 to 6.85 mg/L in T-3.Dissolved oxygen in the beginning was low due to the high organic and nitrogenous load. In the present study, the long duration for the complete bioremediation would have been due to slowing up of the nitrifying process due to the limited aeration supply as nitrifiers are strictly aerobic in nature. The commercial probiotics made from Bacillus subtilis and Bacillus licheniformis improve the water quality parameters to an acceptable range for fish cultivation: 5.7-6.3 mg/L for DO concentration, 0.36-0.42 mg/L for ammonia concentration and p^H between 6.3-8.2 (Haroun et al., 2006). Phosphate-P is one of the major nutrients required for physiological process in living organisms. At the same time it can also be considered as a pollutant leading to eutrophic condition. This study showed 81% reduction of phosphate in T-

3(0.71mg/L).Bhatnagar et al., (2004) stated a safe level of 0.03-2.0 mg/L of phosphate-P as the acceptable range for rearing fishes.

Aquaculture system is deemed to be polluted and unsuitable if BOD and COD levels exceed 10 ppm and 20 ppm (Rath, 2011). BOD and COD reduction was above 90% in T-3 at the end of bioremediation period which showed that the probiotics bacteria were able to oxidize the organic compounds in the waste water more effectively. The variations of pH, dissolved oxygen, alkalinity, hardness, TAN, Nitrate-N, Phosphate-P, BOD and COD were statistically significant ($P<0.01$), between control and test samples. The mixed cultures of Bacillus cereus and Aeromonas vernoii have been identified to effectively degrade fish processing unit effluent, reduce the BOD and Ammonia level (Divya, 2015). Bacillus subtilis, Bacillus cereus, Bacillus mycoides, Pseudomonas sp and Micrococcus sp have shown to be among the best microbes for bioremediation of textile effluents (Mohmood et al., 2013).Naturally occuring consortia of microorganisms like Bacillus, Pseudomonas, Arthrobacter and Micrococcus species could reduce 95% of COD &BOD in steel industry effluents (Krishnaveni et al., 2013).The highest COD reduction was obtained by Coccus and Bacillus 70.7% and 69.5% respectively. The species of Bacillus subtilis and Bacillus licheniformis are best candidate species for bioremediation in a prawn grow out system (Singh, 2002).

The effectiveness of aerobic gram-positive endospore forming bacteria, such as Bacillus sp for improving the water quality by affecting the composition and abundance of water borne microbial populations associated with farmed species was evaluated (Bandyopadhyay and Mohapatra, 2009). The use of commercial microbial products of Bacillus sp increased the quality and viability of pond raised in shrimps (Moriarty, 1998).Bacillus sp were associated with improvement of water quality, reduction of pathogenic Vibrios in culture environment, enhancement of survival and growth rate and improved health status of juvenile penaeus monodon (Nagan and phu 2011). The utilization of food and food conversion efficiency of aquatic prawns can be increased by using probiotics species such as Bacillus subtilis, Bacillus cereus and Bacillus licheniformis (Marrifield et al., 2010).

IV. CONCLUSION

The cellulolytic Bacillus species have shown significant reduction in nitrogenous compounds, phosphorous, BOD and COD of aqua culture ponds making them suitable for rearing of fish and prawns.

V. REFERENCES

[1]. Nikaido H. Multidrug resistance in Bacteria. Annu Rev Biochem. 2009, 78:119-146.

[2]. FAO, 2006. World review of fisheries and aquaculture. The state of world fisheries and aquaculture. Food and agriculture organization of the united nations, Rome, Italy.

[3]. Cutting SM. Bacillus probiotics. Food Microbiol.2011, 28:214-220.

[4]. Gatesoupe FJ. The use of probiotics in aquaculture. Aquaculture.1999, 180:147-165.

[5]. Irianto A, Austin B. Use of probiotics to control furunculosis in rainbow trout, Oncorhynchus mykiss (Walbaum).Journal of fish diseases. 2002, 25(6):333-42.

[6]. Sonia V, Rajagopalswamy CBT, Ahilan B, and Francis T. Influence of bioremediation on the growth and survival of Cyprinus carpio var Koi using aquaculture waste water. Jr. of Industrial Pollution Control.2015, 31(2): 243-246.

[7]. Barak Y, Cytryn E, Gelfand I, Krom M, Rijn JV. Phosphorous removal in a marine prototype, recirculating aquaculture system. Aquaculture.2003, 220:313-326.

[8]. Dalmia G, Kathiresan K, Purushothaman A. Effect of probiotics on bacterial population and health status of shrimp in culture pond system. Indian J.Exp.Biol.2001, 39:939-942.

[9]. Venkateswara AR. Bioremediation to restore the health of aquaculture. Pond Ecosystem.2007, 1-12.

[10]. Porubacan RS. Reduction of ammonia nitrogen and nitrite in tanks of penaeus monodon using floating bio filters containing processed diatomaceous earth media pre inoculated with nitrifying bacteria. World aquaculture society.1991a, 16-20.

[11]. Porubcan RS. Reduction in chemical oxygen demand and improvement in penaeus monodon yield in ponds inoculated with aerobic Bacillus bacteria. World aquaculture society, 1991b, 16-20.

[12]. Xiang- Hong W, Jun L, Wei-shang J, Huai-shu X. Application of probiotics in aquaculture. Ocean University of Qungo. Online.2003, 1-10.

[13]. APHA. Standard methods for the estimation of water and waste water. 22nd edition. American public health association. Washington DC, USA.2012

[14]. Hadi Zokaeifar, Jose Luis Balcazar, Che Ross Sadd, Mohd Salleh Kamarudin, Kamaruzaman Sijam, Aziz Arshad, Naghmeh Nejat. Effects of Bacillus subtilis on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, Litopenaeus vannamei. Fish and shellfish immunology. 2012, 33: 683-689.

[15]. Snecdecor, G.W. and Cochran, W.C. 1967.Statistical methods. Ameslowa, thelowa State University Press.

[16]. Browdy C. Recent developments in penaeid brood stock and seed production technologies: improving the outlook for superior captive stocks. Aquaculture. 1998, 164: 168.

[17]. Bestha lakshmi, Buddolla Viswanath, Sai gopal DVR. Probiotics as antiviral agents in shrimp aquaculture. Journal of pathogens, 2013, ID: 424123:13.

[18]. Albert V, Rasangan J. Effect of water temperature on susceptibility of culture marine fish species to vibrios. International Journal of Research in pure and Applied Microbiology. 2013, 3 (3): 48-52.

[19]. Mohapatra S, Chakraborty T, Prusty AK, Paniprasad K, Mohanta KN. Beneficial effects of dietary probiotics mixture on hemato-immunology and cell apoptosis of Labeo rohita Finerlings Reared at higher water temperatures. PLUS ONE. 2014, 9 (6):100-109.

[20]. Boyd C.E, and Gross, A. Use of probiotics for improving soil and water quality in aquaculture ponds. In Flegel TW (Ed) Advances in Shrimp Biotechnology. National center for Genetic Engineering and Biotechnology, Bangkok, 1998.

[21]. Sahar M, and Asma Khan R. Effect of poultry litter on water Quality and isolation of bacteria from Cyprinus carpio. Periodic Research.2014, 3 (1): 29-34.

[22]. Edwards VA. The nitrogen cycle-Control ammonia, Nitrite in ponds, lakes, Lagoons, Rivers and waste water treatment. 2011. http://www.biosolve.com.au/index_files.

[23]. Sharma A, Pareek B, Review on environmental degradation of petroleum hydrocarbons in marine environment. International Journal of Pharmaceutical Biosciences.2014, 5: 221-227.

[24]. Sarnaik SS, Phalke VV, Kanekar PP.Removal of ammonical nitrogen from fish processing waste water using bioaugmentation technique.Int. J. Pharma.Bio.Sci. 2015, 6 (1):1021-1029.

[25]. Haroun E, Goda A, and Kabir M. Effect of Dietary probiotic Biogen supplementation as a growth promorter on growth performance and feed utilization of Nile tilapia oreochromois niloticus(L). Aquaculture Reasearch. 2006, 37 (14): 1473-1480.

[26]. Bhatnagar A, Jana S.N. Grag S.K. Patra B.C. Singh G.and Barman U.K.Water quality

management in aquaculture. Course manual of summer school on development of sustainable aquaculture technology in fresh and saline waters.2014, 203-210.

[27]. Rath R.K. Fresh water Aquaculture, 3rd Edition. Scientific publishers, india.2011, 478-480.

[28]. Divya M. Isolation, Characterization and biodegradation of potential bacterial strains of seafood processing plant effluent for bioremediation. M.F.Sc thesis. Tamil Nadu Fisheries University, Thoothukudi, 2015, 51.

[29]. Mahmood R, Shariff R, Ali S, Hayyat MU.Bioremediation of textile effluents by indigenous bacterial consortia and its effects on Zea mays L.CVC1415.Journal of Animal and Plant Sciences.2013, 23 (4):1193-1199.

[30]. Krishnaveni R, Pramiladevi Y, Ramgopal Rao S. Bioremdiation of steel industrial effluents using soil microorganisms. International Journal of advanced Biotechnology Research. 2013, 4(1): 51-56.

[31]. Singh BJS. Bioremediation in prawn grow out systems. Technical paper-22, Central Marine Fisheries Research Institute, 2002.

[32]. Bandyopadhyay P. and Mohapatra P.K.D. Effect of a Probiotic bacterium Bacillus circulansPB7 in the formulated diets: on growth, nutritional quality and immunity of Catla catla (Ham).Fish Physiol. Biochem. 2009, 35: 467-478.

[33]. Moriarty DJW, Decamp OP. Probiotics in Aquaculture. AUQA culture Asia Pacific Magazine.2005.

[34]. Nagan P.T.T. and Phu T.Q, Effects of Bacillus bacteria (B8, B37, and B38) on water quality of black tiger shrimp (penaeus monodon) cultured tanks. Proceedings of the 4th aquaculture and fisheries conference.2011, 28-41.

[35]. Merrifield DL, Bradley G, Baker RTM, Davies SJ. Probiotic applications for rainbow trout (Oncorhynchus mykiss Walbaum)II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria post antibiotic treatment. Aquaculture Nutrition.2010, 16 (5):496-503.

Table 1. Concentration and Application of Isolates in Aquaculture Ponds

Treatment	**Isolates Composition**	**Concentration of Isolates culture per gram of feed**	**Pond Application rate**
Control	-	Feed	Per m^3 water
T1	JCEN1,JCEN2,JCEN3&RW	10^5CFU g^{-1} feed	Per m^3 water
T2	JCEN1,JCEN2,JCEN3&RW	10^7 CFU g^{-1} feed	Per m^3water
T3	JCEN1,JCEN2,JCEN3&RW	10^9 CFU g^{-1} feed	Per m^3 water

Table 2. Physico chemical characteristics of raw aquaculture waste water before and after bioremediation.

S. No	Parameter	Before bioremediation	After bioremediation using probiotics			Control
			T-1	T-2	T-3	
1.	Temperature	30^0C	28^0C	28^0C	28^0C	28^0C
2.	pH	8.34±0.008	8.09±0.020	8.08±0.024	8.03±0.014	8.21±0.032
3.	DO	1.08±0.003 mg/L	4.87±0.014	6.32±0.03	6.85±0.008	2.99±0.009
4.	Total Alkalinity	134.80±0.134 g/L	132.43±0.437	132.19±0.389	132.11±0.291	133.31±0.221
5.	Total Hardness	125.60±0.251 mg/L	122.89±0.344	122.81±0.456	122.31±0.324	123.09±0.516
6.	Total ammonia nitrogen(TAN)	0.916±0.001mg/L	0.497±0.0007	0.213±0.0007	0.074±0.0001	0.65±0.008
7.	Nitrite-N	0.0068±0.0001 g/L	0.0048±0.00005	0.0034±0.00001	0.0016±0.00001	0.0055±0.0003
8.	Nitrate-N	0.066±0.0001 mg/L	0.085±0.0003	0.089±0.00002	0.098±0.0001	0.054±0.0001
9.	Phosphate	3.85±0.007 mg/L	2.32±0.003	1.66±0.013	0.71±0.001	2.84±0.0001
10.	BOD	15.63±0.033 mg/L	4.22±0.014	2.24±0.06	1.22±0.012	9.38±0.011
11.	COD	42.63±0.085 mg/L	8.74±0.066	6.28±0.023	2.42±0.014	15.66±0.062