Asian Journal of Agriculture & Life Sciences Vol. 2(4),October 2017: 15-19

Website: www.crsdindia.com/ajals.html e-ISSN: 2455-6149

ORIGINAL ARTICLE

Studies on Bacterial Population from Soil Samples of Shrimp Farm, Nagapattinam, Tamilnadu, India

S. Madhana[1], G. Kanimozhi[1], R. Senthilkumar[2] and A. Panneerselvam[1]

[1] Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi

[2] Department of Microbiology, P.G Extension Centre, Bharathidasan University, Perambalur

Email: madhanaphdmicro@gmail.com

Received: 2nd July 2017, Revised: 8th September 2017, Accepted: 12th September 2017

ABSTRACT

Total 9 different soil samples were collected from shrimp culture farm at Nagapattinam district, Tamilnadu and their physico-chemical properties and population density of bacteria were analyzed. Among them, 7 samples were clay soil; each 1 sample was sandy clay and sandy clay loamy soil. The maximum pH 9 was observed in pond 6 soil sample and minimum 7.8 in pond 3 soil sample. The physiochemical parameters were also analysed. The population density of bacteria was maximum (190×10^5 cfu/ml) and minimum (76×10^5 cfu/ml) observed in clay soil at pond 3 and reservoir. Totally 28 bacterial species were isolated from the nine different samples.

***Key words**: Shrimp farm, soil sample, physico-chemical parameter, Bacteria and population density*

INTRODUCTION

Marine shrimp farming is an aquaculture business for the cultivation of marine shrimp or prawns for human consumption. Although traditional shrimp farming has been carried out in Asia for centuries, large-scale commercial shrimp farming began in the 1970s, and production grew steeply, particularly to match the market demands of the United States, Japan and Western Europe. The total global production of farmed shrimp reached more than 1.6 million tonnes in 2003, representing a value of nearly 9 billion U.S. dollars. About 75% of farmed shrimp is produced in Asia, in particular in China and Thailand. The other 25% is produced mainly in Latin America, where Brazil, Ecuador, and Mexico are the largest producers. The largest exporting nation is Thailand. Shrimp farming has changed from traditional, small-scale businesses in Southeast Asia into a global industry. Technological advances have led to growing shrimp at ever higher densities, and brood stock is shipped worldwide.

The physico-chemical properties of the rearing soil and water are crucial for the success of shrimp culture and the persistent infections could be actually due to poor water quality and low water exchange rates (Zokaeifar *et al.,* 2014). The susceptibility of cultured aquatic species to high concentrations nitrogenous compounds, such as ammonia, nitrite and nitrate, is generally species-specific but high concentrations of these compounds affect animals in aquaculture and likely cause high mortality. The application of gram-positive *Bacillus* species is generally more efficient than the application of gram-negative bacteria species for converting organic matter back to CO_2, which results in the conversion of greater percentage of organic carbon to bacterial biomass or slime (Verschure *et al.,* 2000). The *Bacillus* species enrich the water quality by affecting the composition and abundance of waterborne microbial populations association with farmed species (Bandyopadhyay and Mohapatra, 2009).

Poor water quality in shrimp ponds has been identified as a result of unplanned development of inlet– outlet canals and therefore effluent water discharged from one shrimp farm is often pumped into the adjoining farm. Thus small-scale developers are generally more affected by self-pollution (Jayasinghe, 1999). Since poor water quality is one of the major factors associated with bacterial/vibrio diversity of pond culture systems, assessment of water quality conditions in shrimp ponds is very important to identify the environmental conditions favourable for vibriosis for disease management purposes. Maintaining the pathogenic *Vibrio* load in an aquaculture system below 1000 CFU/ml is very important for which is a proper pond bottom and microbial

management is necessary (Ganesh *et al.,* 2010). Long-term use and misuse of antibiotics may cause alteration of microbial communities and the generation of drug resistance strains of bacteria (Subasinghe *et al.,* 2000).

MATERIALS AND METHODS

SAMPLE COLLECTION:

Soil samples were collected from shrimp farm at Thirupoondi village, Nagapattinam district, Tamilnadu, India. Samples were collected in pre monsoon season during the period of 2015.

ANALYSIS OF PHYSICO-CHEMICAL PARAMETERS OF THE SOIL:

After removing the debris, the soil samples were suspended in distilled water (1:2 w/v) and allowed to settle down the sand particles. The pH of the suspension was determined using pH meter (Systronics, India). Electrical conductivity of the soil was determined in the filtrate of the water extract using Conductivity Bridge as described by Jackson (1973), Cation exchange capacity (CEC) of the soil was determined by using 1N ammonium acetate solution as described by Jackson (1973).

NUTRIENT ANALYSIS:

Organic carbon (OC) content was determined by adopting chromic acid wet digestion method as standard procedure of Walkley and Black (1934), available nitrogen was estimated by alkaline permanganate method (Subbiah and Asija, 1956) and available phosphorus by Bray method (Bray and Kutz, 1945). Available potassium was extracted from soil with neutral 1N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer (Standfold and English, 1949). Calcium (Neutral 1N NH_4 OAC extractable 1:5) was extracted with neutral 1N ammonium acetate and the available calcium in the extract was determined by versenate method (Jackson, 1973). Available micronutrients such as Zn, Cu, Mn were determined in the diethyl triamine penta acetic acid extract of soil (Lindsay and Norvell, 1978), Atomic Absorption Spectrophotometer Perkin-Elmer Model 2280. Other nutrients such as magnesium, sodium and available iron were also analysed (Muthuvel and Udayasoorian, 1999).

ISOLATION AND IDENTIFICATION OF BACTERIA:

1 g soil sample was serially diluted and 0.1 ml diluted sample was inoculated on Zobell marine agar plates by spread plate and pour plate method. After inoculation the plates were incubated at 37±2°C for 48 hours. After incubation period, individual bacterial colonies were identified based on colony morphology, physiological and biochemical characters (Cappuccino and Sherman, 1999).

RESULTS AND DISCUSSION

In the present study, soil samples were collected from nine different shrimp farm in pre monsoon season. Among them, 7 samples were clay soil; each 1 sample was sandy clay and sandy clay loamy soil. The maximum pH 9 was observed in pond 6 soil sample and minimum 7.8 in pond 3 soil sample. Maximum available nitrogen (N) recorded 161 mg/g in pond 5, available phosphorus (P) 7.7 mg/g and potash (K) 240 mg/g were highly in pond 6 soil sample. The higher salinity 5.7% was observed in pond 6 sample lower in pond 1 sample. In micronutrients, maximum zinc 2.50 mg/g, copper 2.58 mg/g, iron 8.25 mg/g, manganese 4.95 mg/g and boron 0.550 mg/g were observed in pond 1, estuaries, pond 4 and 6, pond 6 and reservoir samples respectively (Table 1 and 2).

The population density of bacteria was maximum (190×10^5 cfu/ml) and minimum (76×10^5 cfu/ml) observed in clay soil at pond 3 and reservoir (Table 3). Totally 28 bacterial species were isolated from the nine different samples. Simple correlation co-efficient (r) values were derived for the variables and the results are presented in table 3.

The bacterial diversity of shrimp farm soil in pre monsoon season results were studied. Totally twenty eight different bacterial colonies were isolated in various seasons. Similarly, Heenatigala and Fernando, (2016) reported that 40 bacteria isolates were isolated and identified from shrimp

farm in Sri Lanka. In shrimp culture ecosystems, pathogenic bacteria play a negative role as they compete with shrimps for food and oxygen, while causing stress and diseases (Moriarty 1997). The morphologically, isolated bacterial colonies were observed round, oval, translucent, irregular and the colour was also indicated as orange, yellow, white, pink. The study of marine bacterial diversity is important in order to understand the community structure and pattern of distribution. In the present study, following bacterial isolates were identified as *Acetobacter* sp., *Acidophillus s*p., *Aeromicrobium* sp., *Aeromonas* sp., *Aquaspirillum* sp., *Bacillus* sp., *Bifidobacterium* sp., *Carnobacterium* sp., *Corneybacterium* sp., *Enterococcus* sp., *E. coli, Lactobacillus* sp., *Leuconostoc* sp., *Listeria* sp., *Micrococcus* sp., *Nitrobactor* sp., *Oceanospirillum* sp., *Oscillospira* sp., *Pediococcus* sp., *Planococcus* sp., *Pseudomonas* sp., *Rhodococcus* sp., *Salinococcus* sp., *Shigella* sp., *Staphylococcus* sp., *Streptococcus* sp., *Veillonella* sp. and *Vibrio* sp. (Table 4). Several studies suggested that soil microbial diversity had seasonal fluctuations (Lipson and Schmidt, 2004; Smit *et al*., 1997). Presence or absence of particular bacterial genera may depend on soil parameters, as observed by Alexander (1971).

Table 1: Analysis of physico-chemical properties of soil samples from shrimp farm Nagapattinam

S.No.	Sampling Places	Texture	pH	Bulk density (g/cm^3)	Water holding capacity (%)	Electrical conductivity (dsm^{-1})	Organic carbon (%)	Available Nutrients (Kg/acre)		
								N	P	K
1	Pond 1	Clay	8.60	1.320	29	0.65	0.69	135.0	6.8	180
2	Pond 2	SCL	7.90	1.350	23	0.60	0.74	140.6	6.2	175
3	Pond 3	Clay	7.80	1.150	26	0.78	0.63	125.3	5.9	205
4	Pond 4	Clay	8.50	1.275	30	0.74	0.65	132.4	6.3	215
5	Pond 5	SC	8.90	1.400	28	0.45	0.75	161.0	7.5	230
6	Pond 6	Clay	9.00	1.350	26	0.62	0.70	5.10	7.7	240
7	Before culture	Clay	7.90	1.290	31	0.56	0.76	135.4	5.3	185
8	Estuarie	Clay	8.10	1.170	29	0.49	0.84	145.8	7.4	190
9	Reservoir	Clay	8.20	1.260	28	0.42	0.69	139.6	6.5	220

Table 2: Analysis of available micronutrients of soil samples from shrimp farm Nagapattinam

S.No.	Sampling sites	Salinity %	Available Micronutrients mg/g				
			Zn	Cu	Fe	Mn	B
1	Pond 1	3.6	2.50	1.40	5.10	2.50	0.310
2	Pond 2	4.5	1.85	2.10	5.90	3.25	0.450
3	Pond 3	4.2	1.50	1.15	6.50	3.60	0.425
4	Pond 4	4.1	1.85	2.20	8.25	4.75	0.510
5	Pond 5	5.4	1.95	1.90	7.60	4.10	0.470
6	Pond 6	5.7	2.10	2.25	8.25	4.95	0.535
7	Before culture	4.1	1.65	2.10	6.50	4.56	0.465
8	Estuaries	5.3	1.80	2.58	7.25	4.65	0.490
9	Reservoir	4.3	1.65	2.56	7.30	4.35	0.550

Table 3: Sampling sites and Population Density of Soil

S.No.	Sampling sites	population density (in 10^5) CFU/g of soil
1	Pond 1	79
2	Pond 2	84
3	Pond 3	190
4	Pond 4	98
5	Pond 5	112
6	Pond 6	85
7	Before culture	79
8	Estuaries	128
9	Reservoir	76

Table 4: Biochemical characterization of isolated bacteria

List of Organisms	GS	M	I	MR	VP	CI	TSI	Ca	U	O	NI	CHO Fermentation		
												G	L	S
Acetobactor sp.	(-) rod	Nm	-	-	-	-	K	+	-	-	-	+	+	+
Acidophillus sp.	(+)	M	-	+	-	-	K	+	+	+	-	+	+	-
Aeromicobium sp.	(+) rod	NM	-	-	-	+	K	+	-	-	-	+	-	-
Aeromonas sp.	(-) rod	M	+	+	+	-	A/A	-	+	+	+	+	-	+
Aquaspillium sp.	(-) helical	M	-	-	-	-	K	+	-	+	-	+	-	-
Bacillus sp.	(+) rod	M	-	+	+	+	A/G	+	-	+	-	+	+	+
Bifidobacterium sp.	(+) cocci	NM	+	+	-	+	K	+	-	-	-	+	+	-
Carnobacterium sp.	(+) Cocci	NM	-	-	+	+	G	-	-	-	-	+	-	-
Corynebacterium sp.	(+) rod	NM	-	-	-	+	A	-	-	-	-	+	+	-
Enterococcus sp.	(+) cocci	NM	-	-	+	-	A/G	-	-	-	+	+	-	+
E. coli	(-) rod	M	+	+	-	-	A/G	+	-	-	-	+	+	-
Lactobacillus sp.	(+) cocci	NM	-	+	-	-	K	+	-	-	-	+	-	-
Leuconostoc sp.	(+) cocci	NM	+	-	-	+	K	+	-	-	-	+	+	-
Listeria sp.	(+) rod	M	-	+	+	-	A	+	+	-	+	-	-	-
Micrococcus sp.	(+) cocci	NM	+	-	-	+	K	+	+	-	-	+	+	+
Nitrobactor sp.	(-) rod	M	+	-	-	+	A/A	-	-	+	+	-	+	-
Oceanospirillum sp.	(-) rod	M	-	-	+	-	A/G	-	-	+	+	+	+	+
Oscillospira sp.	(-) rod	M	+	+	-	+	K	+	-	+	-	-	+	+
Pediococcus sp.	(+) cocci	NM	-	+	-	-	A/G	-	+	-	+	-	+	-
Planococcus sp.	(+) cocci	NM	+	+	-	+	K	+	-	-	-	+	+	+
Pseudomonas sp.	(-) rod	NM	-	-	-	+	K	+	+	+	-	+	-	-
Rhodococcus sp.	(+) rod	NM	-	-	-	-	-	+	-	+	+	+	+	-
Salinococcus sp.	(-) Cocci	NM	-	+	+	-	G	+	+	+	-	+	-	+
Shigella sp.	(-) rod	NM	-	+	-	-	-	+	-	-	+	-	+	+
Staphylococcus sp.	(+)cocci	NM	-	-	-	+	K	+	+	-	-	+	+	+
Streptococcus sp.	(+) cocci	NM	-	-	-	+	K	+	-	-	-	+	+	+
Veilonella sp.	(-) cocci	NM	-	-	+	-	A/G	-	+	-	-	+	-	+
Vibrio sp.	(-) rod	M	+	-	-	+	K	+	-	+	-	-	-	+

Note: GS- Gram Staining, M- Motility, I- Indole, MR- Methyl Red, VP- Voges proskauer, CI- Citrate Utilization, U- Urease, O- Oxidase, NI- Nitrate Reduction, G-Glucose, L-Lactose, S- Sucrose, (+)- positive, (-) - Negative, M- Motile, NM- Non Motile, A/G- Alkaline Gas Production, K- Alkaline Production, K/A- Alkaline Acid Production.

REFERENCES

1. Alexander M. (1971): Microbial Ecol., Wiley, New York, USA.
2. Bandyopadhyay P. and Mohapatra P.K.D. (2009): Effect of probiotic bacterium *Bacillus circulans* PB7 in the formulated diets: on growth, nutritional quality and immunity of *Catla catla* (Ham.). Fish Physiology and Biochemistry, 35: 467-478.
3. Bray R.H. and Kutz L.T. (1945): Determination of total organic and available phosphorus in soils. Soil Sci., 59: 39-45.
4. Cappuccino G.G. and Sherman N. (1999): Microbiology a Laboratory Manual Rockland Community College, Suffern: New York.
5. Ganesh E.A., Das S., Chandrasekar K., Arun G. and Balamurugan S. (2010): Monitoring of total heterotrophic bacteria and *Vibrio* sp. In an aquaculture pond. Current Research Journal of Biological Sciences, 2(1): 48-52.
6. Heenatigala P.P.M. and Fernando M.U.L. (2016): Occurrence of bacteria species responsible for vibriosis in shrimp pond culture systems in Sri Lanka and assessment of the suitable control measures. Sri Lanka J. Aquat. Sci., 21(1): 1-17.
7. Jackson M.L. (1973): Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.
8. Jayasinghe J.M.P.K. (1999): Shrimp culture in Sri Lanka: Key issues in sustainability and research. In: Towards Sustainable shrimp culture in Thailand and the Region. Australian Center for International Agricultural Research, Canberra, 48-51 pp.
9. Lindsay W.C. and Norvell A. (1978): Development of a DTPA soil test for zinc, iron, manganese and copper. Proc. Soil Sci. Soc. Am., 42: 421-428.
10. Lipson D.A. and Schmidt S.K. (2004): Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. Appl. Environ. Microb., 70: 2867-2879.
11. Moriarty D.J.W. (1997): The role of microorganisms in aquaculture ponds. Aquaculture, 151: 333-349.
12. Muthuvel P. and Udayasoorian C. (1999): Soil, plant, water are agrochemical analysis. Tamilnadu. Agricultural Unviersity, Coimbatore, India.
13. Smit E., Leeflang P. and Wernars K. (1997): Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. FEMS Microbiol Ecol., 23: 249–261.

14. Standfold S. and English L. (1949): Use of flame photometer I rapid soil test for K and Ca. Agron. J., 41: 446-447.
15. Subasinghe R.P., Barg U. and Tacon A. (2000): Chemicals in Asian aquaculture: Need, usage, issues and challenges. 1-5 pp. In: J.R. Authur, C.R. Lavilla Pitogo, R.P. Subasinghe (eds) Proceeding of the Meeting on the use of Chemicals in Aquaculture in Asia, 20-22 May 1996. SEAFDEC, Tigbauna, Iloilo.
16. Subbaiyah, B.V. and Asija, G.L., 1956. A rapid method for estimation of available nitrogen in soil. Curr. Sci., 25: 258-260.
17. Verschuere L., Rombaut G., Sorgeloos P. and Verstraete W. (2000): Probiotic bacteria as biological agents in aquaculture. Microbiology and Molecular Biology Reviews, 64: 655-671.
18. Walkey A. and Black I.A. (1934): An experimentation of the organic matter and proposed modification of the chronic acid nitration method. Soil Sci., 37:29-38.
19. Zokaeifar H., Babaei N., Saad C.R., Kamarudin M.S., Sijam K. and Balcazar J.L. (2014): Administration of *Bacillus subtilis* strains in the rearing water enhances the water quality, growth performance, immune response, and resistance against *Vibrio harveyi* infection in juvenile white shrimp, *Litopenaeus vannamei*, Fish Shellfish Immunology, 36: pp 68-74.