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Qualitative and quantitative study on bacterial flora of farm raised common carp, *Cyprinus carpio* in India

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Analysis of pond water and sediment as well as skin and intestine of common carp, cultured under polycultre system, was done quantitatively and qualitatively. During the study of 60 days (winter and summer seasons), total viable counts of bacteria were in the range of 4.43±0.50x10^3 to 5.5 ±0.09x10^3 cfu g^{-1} and 7.43±0.03x10^3 to 9.66±0.09x10^3 cfu g^{-1}, respectively in water of A, B and C ponds. In the sediment, bacterial biomass during winter was in the range of 3.23 ±0.06x10^4 to 4.46± 0.15x10^4 cfu g^{-1} and during summer it was found to be in the range of 8.3 0.26x10^4 cfu g^{-1} to 9.43± 0.24 x10^4 cfu g^{-1}. During winter and summer phase, bacterial biomass in skin and intestine was 3.16 0.09 x10^3 cfu g^{-1} to 3.56 0.12x10^3 cfu g^{-1} and 6.03 0.20 x10^5-10^6 cfu g^{-1} to 7.76 0.20 x10^5-10^6 cfu g^{-1} respectively for all 3 replicates. In total, 10 bacterial genera and 13 dominant species were identified. The bacteria of pond water and sediment reflected the bacterial composition in skin, gill and intestine of the fish. In the pond water, *Corynebacterium* spp., *Aeromonas hydrophila, Pseudomonas* spp., *Achromobacter* sp. and *Flavobacter* spp. were predominant whereas in pond sediment, *Aeromonas hydrophila, Pseudomonas* spp*., Corynebacterium* sp., *Flavobacter* spp. and *Bacillus* sp. were predominant and *Corynebacterium* spp., *Aeromonas hydrophila, Flavobacter* spp. and *Pseudomonas* spp. were predominant in skin, gills and intestine of common carp. During the experimental period, *Pseudomonas aeruginosa, Aeromonas hydrophila, Flavobacter devorans and Corynebacterium* sp. were predominantly present in all the samples in all the phases.

Key words: Polyculture, common carp, total viable counts, bacteria sediment, water.

INTRODUCTION

Bacteriology is one of the most important areas determining the pond dynamics and health and hygiene of fish farming system. The present day fish farming is based on nutritive feeds in addition to other management practices thus the bacteriology of cultured fishes in the tropics is receiving greater attention since some species of bacteria associated with fish cause diseases under stress condition. Bacteria within pond environment inhabit the water phase, the bottom sediment and of course live on plants, animals and detritus. Fish is in direct contact

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Table 1. Bacteria biomass (cfu × 10^3) ± S.D. in pond water.

Day	Winter phase			Summer phase		
	Pond A	Pond B	Pond C	Pond A	Pond B	Pond C
0	5.13±0.12	5.2±0.1	4.66±0.59	8.4±0.34	9.26±0.09	7.76±0.24
15	5.20±0.20	5.0±0.21	5.03 ±0.32	8.56 ±0.11	9.33±0.09	7.83 ±0.12
30	4.93± 0.35	4.96± 0.15	4.43± 0.50	8.26± 0.09	8.96± 0.09	7.76± 0.13
45	5.06± 0.24	5.26±0.12	5.26± 0.28	8.16± 0.36	8.86± 0.06	7.43 ±0.03
60	5.1 ±0.43	5.5± 0.09	5.36± 0.26	8.56± 0.20	9.66 ±0.09	7.96 ±0.26

with microflora in the environment and the opportunistic pathogens already present in the water invade the host under stress. There is growing awareness of the influence of bacterial composition of fish, especially in the intestine, on the health and growth of the host (Naim and Ahmed, 2012; Razavilar et al., 2013). Extreme examples of the influence of the gut flora include the negative effects on the pathogenic organisms. The influence of the gut flora on the host is clearly of great interest in aquaculture, particularly where poor productivity and/or stock losses are widespread (Skjermo and Vadstein, 1999; Lavens and Sorgeloos, 2000). The intestinal flora may be of significance in fish spoilage and faecal contamination spread (Al-Harbi, 2003). It is therefore, important to understand the microflora associated with fish culture environment. Recent interest on microbial study of aquaculture products also increases the importance of knowledge of microflora associated with fish (Reilly and Kaferstein, 1997). Bacterial load and bacterial type in shrimp and fish ponds have received attention of researchers recently (Otta et al., 1999; Ahmad and Naim, 2007) but little literature is available on the bacterial flora in cultivable fish (Cahill, 1990; Sugita, 2006). There is limited literature available on microbiological studies in fresh water fish and the culture environment. This study was done to evaluate the normal bacterial counts and identification heterotrophic bacteria found in common carp. The information will be of great value in determining whether there is need to control bacteriological parameters in farming system and also establish relationship between flora of gut of fish and culture water.

MATERIALS AND METHODS

The study was undertaken in the polyculture ponds of 0.3-0.4 ha with a depth of 1.5 m, in the Instructional Fish Farm at College of Fisheries, Pantnagar, India. The ponds were stocked with Catla (*Catla catla*), Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*), Silver carp (*Hypophthalmichthys molitrix*), Grass carp (*Ctenopharyngodon idella*) and Common carp (*Cyprinus carpio*). The ponds of the farm were fertilized regularly and the fishes were daily fed with supplementary feed (rice polish, mustard oil cake and fish meal). Artesian tube well was used regularly to maintain water level. Two trials of 60 days each were conducted in two major seasons, that is, winter (December-January) and Summer (April - May). Soil and water sampling was done fortnightly. The samples were collected from three spots in the pond and mixed together. Water was analyzed for some important physico-chemical parameters using standard techniques (APHA, 2005). Skin and intestine samples were collected fortnightly, slaughtering three fishes at each sampling. The plate count method was used for quantitative estimation of aerobic heterotrophic bacteria from pond water, sediment and fish using plate count agar media (Breed et al., 1957). The bacterial isolates were identified to genera and species level by morphological studies, staining procedures and bio-chemical and physiological tests, that is, catalase test, acid and gas production from carbohydrates, starch hydrolysis, gelatin hydrolysis, decarboxylation of amino acids, indole production, Methyl Red and Voges-Proskauer (MRVP) test, urea hydrolysis and H_2S production (Breed et al., 1957). The data were analyzed by one way ANOVA (Panse and Sukhame, 1978). All the data were found significant at 5%.

RESULTS

The results of total bacterial counts carried out at 30±2°C for samples of experimental pond water, pond sediment and experimental fish skin and intestine are recorded in the Tables 1, 2, 3 and 4, respectively. It is clear from these values obtained from bacterial counts that samples are significantly different and observations showed seasonal variation too. The results of total plate count of pond water of pond A, during winter season, were found in the range of 4.93±0.35x10^3 to 5.2±0.2x10^3 cfu ml^{-1}; for pond B 4.90 ±0.15x10^3 to 5.5±0.09x 10^3 cfu ml^{-1} and 4.43±0.15x10^3 to 5.36 ±0.09x 10^3 cfu ml^{-1} for pond C (Table 1). During the summer phase counts of bacteria were in the range of 8.16±0.36x10^3 to 8.56±0.2x10^3 cfu ml^{-1} in pond A, 8.86±0.36x10^3 to 9.66±0.09x10^3 cfu ml^{-1} in pond B and 7.43±0.30x10^3 to 7.96±0.26x10^3 cfu ml^{-1} in pond C (Table 1). The heterotrophic populations in the sediment were in the range of 3.46±0.15x10^4 to 4.06±0.15x10^4 cfu g^{-1} in pond A, 3.80±0.27x10^4 to 4.46±0.15x10^4 cfu g^{-1} in pond B and 3.23±0.06x10^4 to 4.08±0.07x10^4 cfu g^{-1} in pond C (Table 2) during winter phase whereas during summer season, counts of bacteria were in the range of 8.3±0.26x10^4 cfu g^{-1} to 9.3±0.18 x10^4 cfu g^{-1} in pond A, 8.53±0.06x10^4 to 9.43±0.24x10^4 cfu g^{-1} in pond B and 8.23±0.09x10^4 to 8.53±0.22x10^4 cfu g^{-1} in pond C. Bacterial biomass on the body of the skin of the fish showed marked difference at the skin and in the intestine. Total counts were always higher in the intestine whereas skin bacterial counts were lower in the seasons, winter as well as summer. Table 3 shows that during winter season, the total viable counts of bacteria in

Table 2. Bacteria biomass (cfu × 10^4) ± S.D. in pond sediment.

Days	Winter phase			Summer phase		
	Pond A	Pond B	Pond C	Pond A	Pond B	Pond C
0	3.83 ±0.11	3.96 ±0.12	3.26 ±0.12	8.3± 0.26	8.53 ±0.06	8.13 ±0.09
15	3.53± 0.12	4.1± 0.12	3.45 ±0.12	8.6 ±0.06	9.2± 0.19	8.46 ±0.09
30	3.46 ±0.15	3.8 ±0.27	3.23 0.06	9.25± 0.15	9.25± 0.09	8.46 ±0.09
45	3.76 ±0.18	4.26 ±0.03	3.46 ±0.05	8.6 ±0.07	8.5 ±2.89	8.23 ±0.09
60	4.06 ±0.15	4.46± 0.15	4.08 ±0.07	9.3± 0.18	9.43± 0.24	8.53± 0.21

Table 3. Bacteria biomass in skin (cfu × 10^3 cm^{-2}) ± S.D. and intestine (cfu × 10^5- 10^6 g^{-1}) ±S.D. of common carp.

Days	Pond A		Pond B		Pond C	
winter phase	Skin	Intestine	Skin	Intestine	Skin	Intestine
0	3.23± 0.09	6.53± 0.22	3.32 ±0.06	7.76± 0.15	3.43±0.09	6.16±0.12
15	3.43± 0.18	6.76± 0.19	3.46 ±0.15	7.63 ±0.15	3.26±0.09	6.5 ±0.15
30	3.2 ±0.12	6.53 ±0.18	3.26± 0.12	7.6 ±0.23	3.16±0.06	6.03±0.20
45	3.3± 0.21	6.66 ±0.15	3.56± 0.12	7.66 ±0.33	3.43±0.12	6.53±0.13
60	3.46± 0.12	6.9± 0.12	3.73 ±0.03	7.76 ±0.20	3.56±0.12	6.6 ±0.12
Summer phase						
0	8.2 ±0.09	1.87 ±0.37	8.2± 0.09	2.19 ±0.07	7.93±0.09	1.85±0.04
15	8.33± 0.21	1.89± 0.48	8.33± 0.21	2.21± 0.01	8.33±0.09	1.87±0.02
30	8.0 ±0.15	1.85± 0.38	8.0 ±0.15	2.19 ±0.12	8.13±0.13	1.86±0.01
45	9.16 ±0.20	1.86± 0.37	9.16 ±0.20	2.16± 0.08	7.93± 0.1	1.82±0.03
60	9.53± 0.11	1.90 ±0.39	9.53 ±0.11	2.22± 0.01	8.4± 0.12	1.88 ±.03

Table 4. Percentage incidence of bacteria from pond water, sediment and fish (skin and intestine).

No. of isolates / Bacteria	Incidence (%)			
	90	118	60	78
	Water	Sediment	Skin	Intestine
Pseudomonas spp.	49	50	36	25
Aeromonas sp.	18	21	12	30
Flavobacter spp.	5	4	5	4
Achromobacter sp.	3	4	1	1
Corynebacteria spp.	3	5	2	3
Vibrio sp.	2	3	2	3
Proteus sp.	1	4	1	-
Micrococcus sp.	2	-	-	-
Bacillus sp.	-	3	-	-

the fish skin ranged from 3.2±0.12x10^3 to 3.46±0.12x10^3 cfu cm^{-2} for pond A, 3.26±0.12x10^3 to 3.73±0.03x10^3 cfu cm^{-2} for pond B and 3.16±0.06x10^3 to 3.56±0.12x10^3 cfu cm^{-2} for pond C. During summer season, bacterial load in the skin of fish (Pond A) ranged from 8.0±0.15x10^3 to 9.53±0.12x10^3 cfu cm^{-2}, 8.49±0.09x10^3 to 9.3±0.19x10^3 cfu cm^{-2} for pond B and 7.93±0.09x10^3 to 8.4±0.12x10^3 cfu cm^{-2} for pond C. The bacterial counts showed lower numbers in winter phase as compared to summer phase. During winter season, the total viable counts of bacteria in the fish intestine ranged from 6.53.2±0.18x10^5 to 6.9±0.12x10^5 cfu g^{-1} for pond A, 7.6±0.23x10^5 to 7.76±0.2x10^5 cfu g^{-1} for pond B and 6.03±0.20x10^5 to 6.60±0.12x10^5 cfu g^{-1} for pond C (Table 3). Total aerobic plate counts for intestine of fish in different ponds during summer phase were 1.85±0.38x10^6 to 1.9±0.39x10^6 cfu g^{-1} for pond A, 2.16±0.01x10^6 to

Table 5. Identity of the bacteria with source of isolation.

Source of sample	Identity of the bacteria
Pond water	*Corynebacterium* spp., *Pseudomonas aeruginosa*, *P. fluorescens*, *P. aureofasciens*, *Aeromonas hydrophila*, *Flavobacter devorans*, *Proteus* sp., *Micrococcus* sp.,
Pond sediment	*Corynebacterium* spp.,*Ps.aeruginosa*, *P.fluorescens*, *P.aureofasciens*, *Aeromonas hydrophila*, *Flavobacter devorans*, *Bacillus* sp., *Proteus* spp., *Achromobacter* sp.
Fish skin	*Corynebacterium* spp., *Aeromonas hydrophila*, *Flavobacter devorans*, *Achromobacter* sp., *Pseudomonas aeruginosa*, *Vibrio* sp., *Proteus* sp.
Fish intestine	*Corynebacterium* spp., *Aeromonas hydrophila*, *Flavobacter devorans*, *Pseudomonas aeruginosa*, *Vibrio* sp., *Achromobacter* sp.

$2.22\pm0.01x10^6$ cfu g^{-1} for pond B and $1.82\pm0.03x10^6$ to $1.88\pm0.03x10^6$ cfu g^{-1} for pond C. Bacterial profile of pond and fish (Table 4) reveal that *Pseudomonas* spp. and *Aerobacter* sp. were the dominant groups. In rearing water and pond sediment, *Pseudomonas* spp. was the dominant flora, comprising about 27-51% of the total population followed by *Aerobacter* sp. with 12-31% of the total population. The predominant organisms associated with the fish skin were the *Pseudomonas* group, which comprised 41 and 38% of the total bacteria observed. In the fish gut, *Aeromonas* sp. was dominant (31%) while *Pseudomonas* comprised 27% of the total isolates. The isolates from pond water were identified as *Corynebacterium* spp., *Aeromonas hydrophila, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas aureofasciens, Vibrio sp., Flavobacter devorans, Proteus* sp. and *Micrococcus* sp. In pond sediment, *A. hydrophila, Pseudomonas* spp. *Corynebacterium* spp., *Bacillus* sp., *Flavobacter devorans, Achromobacter* sp. and *Proteus* sp. were present. In total, 11 species belonging to nine genera were identified from pond water, sediment and fish. In addition to the above bacterial species, *Vibrio* sp. was also observed in the fish (skin and intestine). The distribution of these bacteria in the five type of samples, that is, pond water, pond mud, skin, gill and intestine of fish are shown in Table 5.

DISCUSSION

Ayyappan and Pande (1989) reported the bacterial population in water phase in Indian ponds in the range of 0.12 to 4.65×10^3 no./ml. Das and Mukherjee (1999) found the bacterial counts in the range of 0.8-25.2 × 10^3 cfu/ml in polyculture ponds. The mean total viable counts of the pond water recorded, was 1.8x103-4.5x 10^3 cfu ml^{-1} during the study period in a semi intensive pond by Sharmila et al. (1996). In the present study, the bacterial load recorded for the two ponds were within the limits as reported by the previous workers. The heterotrophic bacterial population in the sediment was in the range of 0.79-26.67 × 10^4 nos/g in culture ponds of India, as reported by Ayyappan and Pandey (1989). Ayyappan and Pandey (1989) recorded total viale cell counts ranging from $1.0x10^5$ to $6.2x10^5$ cfu g^{-1} for the pond sediment. On the basis of the above findings, it can be stated that the level of sediment bacteria recorded in the present study was within the range. Bacterial density was higher during summer phase as compared to winter phase. This is obvious because of the ambient temperature leading to higher rate of metabolic activities and growth. Bacterial density was observed to be ten times higher at the pond sediment than in the water medium. This is expected because organic matter content is greater at the pond as the total solids of manure are suspended. Similar observations were recorded by other workers also (Glucai et al., 1992; Al-Harbi and Uddin 2004). Total bacterial counts were maximum in the intestine in all the three phases while skin showed minimum bacterial load. The observations are also supported by the findings of Ahmad and Naim (2007) where total bacterial load was in the range of $3.4\pm 1.8 \times 10^6$ to $5.8\pm0.4 \times 10^7$ cfu g^{-1} in the intestine and $7.1\pm 0.7x10^5$ to $8.7 \pm 1.1x10^6$ cfu g^{-1} in the gills. *Pseudomonas* spp. predominated in all the bacterial isolates examined from pond water and sediment. The finding is supported by previous reports (Fasanya et al., 1988; Das, 1991). *Aeromonas hydrophila* followed *Pseudomonas* in water and sediment (51 and 21% respectively in water and 46 and 19% respectively in the sediment). All the bacterial genera identified in skin, gill and gut were represented in water although in different percentages of incidence. Previous workers (Roberts, 1978; Sakata et al., 1980; Sugita et al., 1988; Das and Mukherjee, 1999; Ahmad and Naim Uddin, 2007) have reported that bacterial flora observed on surface, gill and intestine of pond reared fish/prawn are a reflection of bacterial flora of pond water. *P. fluorescens, Aeromonas hydrophila, Edwardsiella tarda, Vibrio* spp. and

Myxobacteria have been reported as normal microflora of water by Naim Uddin and Ahmed (2012) and Razavilar (2013) and can be found on body surface or in the intestinal tract of fishes (Gary, 2005). These bacteria under environmental stress produce epizootic outbreaks. According to Cristopher et al. (1978), Bacillus, *Corynebacteria, Flavobacter* and *Vibrio* are other dominant flora with *Pseudomonas*. *A. hydrophila* was the most dominant isolate in intestine and gills of common carp, with 31 and 20% incidence, respectively. This organism is one of the most opportunistic pathogens for fresh water fish as reported by Das and Mukherjee (1999). Aeromonads were the main etiological agents in disease outbreaks in India where several mortalities have been recorded (Kumar, 1989; Das, 1991; Nayak, 1993). During all the three phases, *P. aeruginosa, A. hydrophila, F. devorans* and *Corynebacteria* sp. were predominantly present in all the samples. Mesophilic aeromonads have been reported by several workers (Campbell and Buswell, 1983; Sugita et al., 1988) in wild and aquaculture freshwater fish and prawn. It is evident from this preliminary study that bacterial types isolated from *C. carpio* and its environment were all aerobic heterotrophic. Diverse genera of Gram negative bacteria were detected. The potential pathogenic nature of Gram negative isolates (*A. hydrophila, P. fluorescens* and *Vibrio* sp.) is beyond doubt as several reports support (Roberts, 1972; Sugita et al., 1988; Otta, 1999). It may be concluded that this study shows that there are several potential pathogens present in the environment and also as part of normal bacterial flora in Indian major carp common carp. Under stressful conditions, these organisms may become opportunistic and attack the body tissue and produce disease. The need is thus felt to monitor and regulate the bacterial parameters in the present aquaculture system where lot of management is done to enhance production.

Conflict of interests

The author(s) have not declared any conflict of interests.

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