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Isolation and Characterization of Pathogenic Bacterial Flora Associated with Some Ornamental Fishes of Jammu (J&K)

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Studies on the disease infestation of fish pathogen isolated from fishes *Trichogaster fasciatus* (Bloch.), *Channa punctatus* (Bloch.) and *Esomus danricus* (Ham.) collected from a stream water in R.S. Pura Jammu was conducted. Inoculum taken from the skin, gills and intestines indicated the presence of a variety of aquatic bacteria. A total of twenty-two isolates were identified phenotypically, of which fifteen isolates were found to be pathogenic. Percentage incidence of different pathogens i.e., *Salmonella* spp. was 53.3%, followed by 26.66% of *Escherichia coli,* 13.33% of *Pseudomonas* spp. (causing skin lesions) & 6.66% of *Proteus vulgaris (*causing Red Spot disease*) in Channa puctatus* and *Trichogaster fasciatus* respectively. Of the three fishes investigated, percental occurrence of pathogenic infection was significantly higher (P<0.005) in *Channa punctatus*. However, *Trichogaster fasciatus* shows the presence of only *Proteus vulgaris* infection.

Key words: Pathogen, Ornamental fishes, Isolates, Skin lesion.

Bacterial diseases contribute to natural mortality and can be causative factors for growth retardation in fishes. Several species of bacteria are opportunistic pathogen and produce disease when they are under stressed conditions caused due to aquatic pollution (Sharma 1993; Dalsgaard & Madsen, 2000; Al – Harbi & Uddin, 2003 and Hudson & Peters, 2005).

It is surprising that little attention has been given to the ornamental fishes since large numbers of these fishes are procured from the areas where sanitation is often inadequate. Since, many of these fishes are offered for sale to the public, who mainly purchase them for edible purpose apart from using them as aquarium fishes (Roos *et al*., 2000, 2003). It is apparent that the presence of potentially pathogenic micro-organisms in these fishes would present a risk to the public health causing infections of eyes, ears, nose, throat and urino – genital and gastric system (Bailey & Scott, 1966; Krieg & Holt, 1984 and Talaro & Talaro, 1996).

Presently an attempt has been made to investigate qualitative data on disease causing pathogens in some local ornamental fishes of Jammu region.

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MATERIAL AND METHODS

Fishes viz., *Channa punctatus*, *Esomus danricus* and *Trichogaster fasciatus* were collected from a side pool of stream in R.S.Pura, Jammu region. Then Inoculum from fish skin, gills and gut were taken under aseptic conditions and streaked on nutrient agar slants for studying the growth patterns at 37° C for 24 hours. The culture was then transferred to Mc Conkey's broth, Brain heart infusion, Soyabean casein digest broth and Salenite broth for enrichment at 37° C for 24-48 hours again. The bacteria were later sub-cultured on Mc Conkey's agar, Citramide agar, Deoxycholate citrate agar (DCA) and Eosin-methylene blue (EMB) agar plates at 37° C for 24-48 hours. The colonies formed were restreaked to get single pure colony. Identification of the isolated micro-organisms were performed by microbiological and biochemical assays. For bacterial identification biochemical tests were made according to James and Natalia, (1999) and their characteristics were compared with those given in the Bergey's manual of systematic bacteriology (Krieg & Holt, 1984).

The characteristics of colony were recorded depending upon its size, shape, and pigment production on agar plates and morphology studied by Gram's staining. Biochemical Characteristics were performed such as triple sugar-iron agar test (TSI), catalase reaction, nitrate reduction, indole production, oxidase test, citrate and urea utilization, gelatine liquification test, methyle-red and vogus-proskaur reaction. The identification of the isolates was done on the basis of biochemical tests and further confirmed by PCR technique. PCR was carried out using primers as described below :

QVR 184 5' ACGCATTTTGCGTTTATTCC 3'
QVR 185 5' GGATTGCCTGGCTCATAAAC 3'
for *Salmonella* flg B gene
QVR 201 5' GGCTTCTGTCAACGCTGTTT 3'
QVR 202 5' ACAGTTTTCGCGATCCAGAC 3'
for *E.coli* vid A gene and 100 bp & 1 kb DNA ladders were used as DNA size marker.

RESULTS AND DISCUSSION:

All the bacteria isolated from different regions viz., skin, gills and intestines were found to be Gram-negative anaerobic rods. From the results it was revealed that out of twenty-two isolates, fifteen were pathogenic isolates belonging to the different genera. Maximum number of bacteria were isolated from the skin. Infections caused by *Salmonella* spp. was found to be predominant (Fig. 6) i.e. 53.3%, followed 26.66% of *Escherichia coli,* 6.66% of *Pseudomonas* spp. & 6.66% of *Proteus vulgaris*. Samples taken from skin, gills and intestines were inoculated on different agar plates and were partly identified by their differentiating properties i.e. formation of black colonies on Deoxycholate citrate agar (DCA) plates in case of *Salmonella* spp., green metallic sheen on Eosin-methylene blue (EMB) agar plates in case of *Escherichia coli,* and *Pseudomonas* spp. was identified by its characteristic florescent green pigmentation on Citramide agar plates. A characteristic zonation pattern of successive waves, separated by periods of quiescence and growth clearly distinguished the *Proteus vulgaris* from other pathogenic bacteria.

In addition to these culture characteristics, various biochemical tests were also performed to confirm the different pathogens. Eleven phenotypic traits examined to determine the particular pathogenic bacteria. As per the results only *Salmonella* spp. found to produce hydrogen sulphide gas with acid butt and alkaline slant while rests of the three pathogens were incapable of producing hydrogen sulphide gas. All the results of the biochemical tests were found to be similar as cited in Bergey's Manual of Systemic Bacteriology (1984). Further the PCR reaction carried out in *Salmonella* and *E.coli* revealed the amplification product of 752 & 293 base pairs (Fig. 4 & 5).

In *Channa punctatus* (6.5 ± 0.5 cm) nine isolates were taken out of which three were found to be of *Salmonella* spp., four of *Escherichia coli* and two of *Pseudomonas* spp. while *Proteus vulgaris* was not detected. In *Esomus danricus (2.5* ± 0.2 cm) all the five samples taken were found to be of *Salmonella* spp. In *Trichogaster fasciatus* (5.8 ± 0.2 cm) out of total eight samples, only one case of *Proteus vulgaris* was detected.

Sharma (1993) studied the qualitative and quantitative bacterial flora of the fishes in relation to that of a pond in IIIM, Jammu. Mohamed and Lakshmanaperumalsamy (1997) and Aweeda *et al*.

(*Channa punctatus*)

Fig. 1. Skin lesion

(*Trichogaster fasciatus*)

Fig. 2. Red Spot disease

Fig. 3. Enlarged portion of skin lesion (*Channa punctatus*)

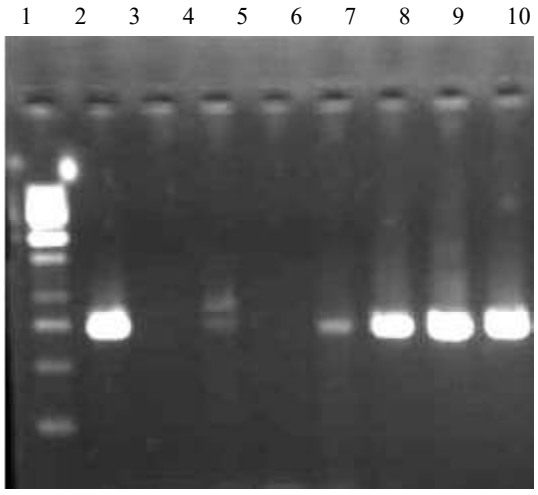

Fig. 4. 1% Agarose gel showing the detection of *Salmonella* isolated from fish with primers QVR184 - QVR185

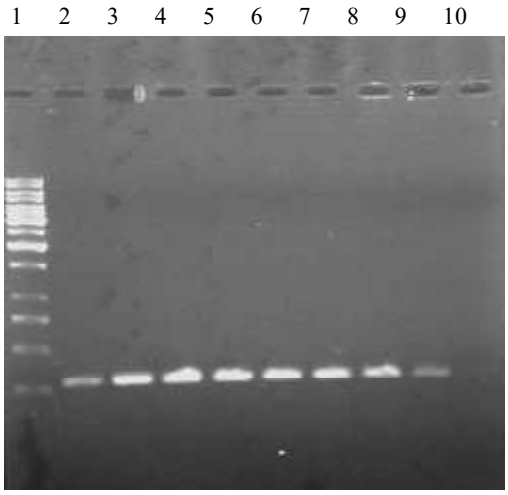

Fig. 5. 1% Agarose gel showing the detection of *E. coli* isolated from fish with primers QVR201 - QVR202

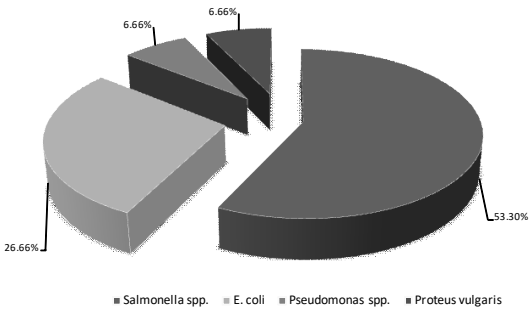

Fig. 6. Pie chart showing the percentage occurrence of fish pathogens in all fishes

(2006) in their study on the prevalence of *Salmonella* spp. in fish and crustaceans from different areas, found high count of *Salmonella* spp. in samples from gut of fishes and gills of crustaceans. And one of the possible reasons behind the predominance of *Salmonella spp*. could be the faecal contamination of water as observed by many workers (Bailey & Scott, 1966 and Polo *et al.,* 1999).

During the present investigation, two cases of skin lesions in *Channa punctatus* (Fig.1 & 3) and one of Red Spot Disease (RSD) in *Trichogaster fasciatus* (Fig.2) were recorded. *Pseudomonas* spp. was isolated from all the samples of skin lesions in *Channa punctatus*. The present findings are in lined with various workers (Manohar *et al.,* 1976; Kumar *et al.,* 1986; Karunasagar *et al.,* 1988 and Sahu *et al.,* 1966). However secondary infections of *Salmonella* spp. and *Escherichia coli* were also found in the skin lesion samples during the present investigation. Red spots disease in *Trichogaster fasciatus*, showed the presence of *Proteus vulgaris*. Van Duijn (1956) also reported similar findings in the same fish species. Exophthalmus in *Ophiocephalus punctatus* (Chawadhary *et al.,* 1978) and Cauliflower disease in *Rasbora daniconius* (Srivastava *et al.,* 1978) were reported so far in Jammu region.

The present investigations has shown the high percentage of known fish pathogens, predispose the local ornamental fishes to bacterial epizootics, especially if populations are stressed by poor environmental conditions or by environmental contaminants.

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