Investigation into the Virulence of Psuedomonas fluorescens associated with the diseased fresh water Cirrhina mrigala

Ravi D. Barde
Department of Zoology, SGB College, Purna, Maharashtra, India,
ravibarde4u@gmail.com

Abstract

In India, fish bacteriology has a slow progress when compared to developed countries. The bacterial diseases reported so far are dropsy or hemorrhagic septicemia, fin rot, ulcer disease, columnaris disease, eye disease of Catla, skin disease of Catfish, stereptococcal infection etc Among the Indian Major Carps are Labeo calbasu, Cyprinus carpio, Cirrhinus mrigala, Catla catla and Labeo rohita. Cyprinus carpio and Cirrhinus mrigala are the second fast growing and are widely consumed throughout India.

Cirrhinus mrigala is susceptible to bacterial diseases in natural as well as cultured system and is infected with Pseudomonas fluorescens. The survey revealed the occurrence in the region of Marathwada in most of the waterbodies surveyed. Two most virulent isolate among the isolated strains were tested for their ability t cause symptoms and subsequent death in the experimental fishes. The preliminary tests indicated that ulcers were associated with Pseudomonas fluorescens PF-02 and PF-14 isolates. The LD50 values were estimated as 0. 971 X 105, and 0.901 X 105 for isolates PF-02 and PF-14, respectively.

Keywords: *Cirrhinus mrigala, Pseudomonas fluorescens, LD 50.*

INTRODUCTION

Diseases caused by bacterial are associated with substantial death in both wild and cultured fishes. The authentic part of these pathogenic microorganisms may vary from a prime pathogen to that of an opportunistic pathogen that imparts its host decline by initiating a disease process (Barde, 2021).

Infection in fishes brings about deleterious effects in their growth, resistance to stress and fecundity, and in addition, they are vulnerable to predation and sub optimal environmental factors. All these variables decimate the fish, indirectly impinging a heavy economic loss to the fish farmers, and it becomes imperative for them to control the infection by constant surveillance and development of adequate control measures to prevent spreading of diseases and to promote healthy environment in all the farms.

It is common knowledge that cultivable fishes are susceptible to disease-causing pathogenic bacteria (Darak & Barde, 2014; Darak et al.,2021) and fungi (Barde et al., 2020, 2021). Bacterial diseases of fish have always caused night mares among fish farmers. Among bacterial diseases, furunculosis, ulcer disease, columnaris disease and several others have caused epizootics of such magnitude as to threaten the entire salmonid fisheries of the affected regions. Furunculosis of salmonid fish received more attention from pathologists, and has been a serious problem in hatcheries of many countries, like USA, Germany, Italy, Canada and UK. It is the first bacterial fish disease which has been worked out fully.

In India, fish bacteriology has a slow progress when compared to developed countries. The bacterial diseases reported so far are dropsy or hemorrhagic septicemia, fin rot, ulcer disease,

columnaris disease, eye disease of Catla, skin disease of Catfish, streptococcal infection etc .(Darak and Barde, 2015) Therefore, it is necessary to throw emphasis on induced infections and to develop suitable control methods.

The Indian major carps are the most important food fishes and they are of very high commercial value. They are widely cultivated all over India, especially in central and South India and particularly in Tamil nadu. Private entrepreneurs have undertaken large scale fish farming by converting many a rice field into commercially viable fish farms. Among the Indian Major Carps are Labeo calbasu, Cyprinus carpio, Cirrhinus mrigala, Catla catla and Labeo rohita. Cyprinus carpio and Cirrhinus mrigala are the second fast growing and are widely consumed throughout India. Cirrhinus mrigala is susceptible to bacterial diseases in natural as well as cultured system. Hence, it was chosen as the experimental organism to find out the effects of pathogenic bacterial strain on the physiology and nutritional value of the fishes following infection by bacterial pathogen.

Material and methodology:

Fish Samples

Fish samples of fish species Cirrhinus mrigala species had been collected typically from surrounding natural bodies of water and cultivated systems. After determining the source, some fish were also bought from the local markets for this investigation. Samples were taken for analysis after processing at the laboratory immediately. Immediately after catching with sterilized cotton swabs, samples of different body parts were obtained. Within 4 hours of selection the samples were cultivated. The fishes were selected and considering their easier availability, the health status of the fishes was given high priority.

Isolation and Maintenance of cultures

The isolation of heterotrophic, aerobic and anaerobic bacterial communities in terms of cfu /ml were identified and enumerated by the standard methods described by Cheesbrough (1989) and Bergey's Manual for Systematic Bacteriology (1986). Cultures were preserved by asceptically transferring the bacterial cultures to freshly prepared sterile nutrient agar slants after every three weeks. The stock cultures were stored at 4°C. For experimental works, subcultures were made from the stock cultures in suitable media before use. The cultures were also examined at regular intervals to test their pathogenicity.

Determination of LDW values of the pathogenic bacteria

The two pathogenic bacteria, Aeromonas spp. (PF-02 and PF-14) were grown in Brain Heart Infusion Broth at 30°C for 48 hours. These cultures were used to make five 10 fold dilutions of calculated by the spread plate method. 0.05 ml of each of these diluted cell suspensions were injected intraperitoneally to 10 healthy fishes. The doses received by the fishes ranged from 1x103 to 1x107 cfu. Each of the 10 control fishes received 0.05 ml of sterile 0.85% NaCl solution. Following injection, the fishes were observed for a 15 days period to record the appearance of disease symptoms and mortality. The dead fishes were immediately sacrificed and parts of liver and kidney were incubated in nutrient broth for re-isolation of the bacteria. The lethal dose 50% end, (LD50) was calculated from the relationship between the probits of percentage mortalities and the logs of the dilution series of bacterial suspension.

Result:

Isolation and maintenance of Pseudomonas fluorescens from various location of Marathwada region

A Total of eighteen (18) isolates of Pseudomonas fluorescens were isolated from eight different location of Marathwada districts. 04 isolates each were isolated from Nanded district, 02 isolates each were isolated from Parbhani, Hingoli, Latur, Beed, Osmanabad, Jalna and Aurangabad districts each (Table 1). These isolates were identified and maintained on their characteristics of growth on Nutrient agar slants.

Table1: Isolate codes of Pseudomonas fluorescens with their location of isolation

Sr. No	District	Natural culture system	Isolate code of *Pseudomonas fluorescens*	Artificial culture system	Isolate code of *Pseudomonas fluorescens*	Total
1	Nanded	Godavari (Vishnupuri)	Pf-01	Kandhar	Pf-03	04
		Asna (Devapur)	Pf-02	Petwadaj	Pf-04	
2	Hingoli	Kayadu (Balapur)	Pf-05	Bhategaon	Pf-06	02
3	Parbhani	Masoli (Gangakhed)	Pf-07	Yeldari	Pf-08	02
4	Aurangabad	Nagzhari (Tembapuri)	Pf-9	Paithan	Pf-10	02
5	Jalna	Galhati (Ambad)	Pf-11	Ghanewadi	Pf-12	02
6	Latur	Manjra (Latur)	Pf-13	Sakol, Udgir	Pf-14	02
7	Beed	Sindphana (patoda)	Pf-15	Mehakari, Ashti	Pf-16	02
8	Osmanabad	Sina (Paranda)	Pf-17	Khasapur	Pf-18	02
			09		09	18

Pathogenicity Studies of two Pseudomonas (PF-02 and PF-14) isolates in fish ulcer disease using Cirrhinus mrigala

Two isolates Aeromonas (PF-02 and PF-14) were purified from the skin ulcers of the fish concerned. Since these bacteria were isolated from the fish's ulcer tissue, it was considered logical to study the clinical symptoms, histopathology and hematology of fish infected with these pathogenic bacteria. The study of the pathogenicity and virulent characteristics of these bacteria were studied in detail.

Pathogenicity studies of the bacteria PF-02 and PF-14

The preliminary tests indicated that ulcers were associated with PF-02 and PF-14. It was thought proper to determine the amount of inoculum required to cause symptoms in healthy fishes. There are little records of these bacteria's LD50 values in any fish.

Therefore, experiments were conducted to establish the LD50 values of these bacteria, and a comparative study on the sensitivity of these fish species to these pathogenic bacteria was carried out.

Determination of the LD50 value

Bacteria isolates PF-02 and PF-14 cell suspensions were injected into healthy fish in serially diluted doses ranging from 1X102 to 1X107 cfu in 0.05 ml of inoculum. Materials and Methods gives details of procedures followed. Fish mortality rates increased with all bacterial suspensions with increasingly concentrated. The bacterial isolates PF-02 and PF-14 could be purified from the dead fish lesion of liver and kidney. At the highest concentration (1x107 cfu), 85% of the fish inoculated with PF-02 died and PF-14 died at the lowest concentration (1x103 cfu). The LD50 values were estimated as 0. 837 X 105, and 0.878 X 105 for isolates PF-02 and PF-14, respectively (Table 2).The inoculums concentration 1X102 did not induced death or symptoms in fishes.

Table 2: Cumulative mortalities of Cirrhinus mrigala after 15 days of inoculation with serial 10 fold dilutions of PF-02 and PF-14 (Pseudomonas fluorescens).

Dose (c.f.u.)	Number of fishes inoculated	Number of dead fishes	
		PF-02	PF-14
1 X 10^2	20	0	0
1 X 10^3	20	3	4
1 X 10^4	20	6	5
1 X 10^5	20	10	9
1 X 10^6	20	11	11
1 X 10^7	20	15	14
LD50		0. 971 X 10^5	0.901 X 10^5

c.f.u 0.05 ml of inoculum

b Calculated number of bacteria required to kill 50% of injected fish. (Calculated from relationship between probits of percentage mortalities and the logs of the dilution series of bacterial suspension).

Comparative mortality rate of the two fish species

The mortality rate was 65.0% when inoculated with a mixed bacterial suspension of PF-02 and PF-14 in Cirrhinus mrigala. The pure bacterial suspensions, PF-02 induced 35 % and PF-14 induced 40 % mortality (Table 3).

Table 3: Percentage mortality & Nature of ulcer formation in Cirrhinus mrigala injected intramuscularly with saline suspensions of PF-02 & PF-14 in pure & mixed condition.

Bacterial strain	*Number of fishes inoculated*	*Number of fishes dead*	*Nature of ulcer*	*Percentage mortality*
Control	20	0	-	-
PF-02	20	8	moderate	40.0%
PF-14	20	7	moderate	35.0%
Mixed	20	15	severe	75.0%

Control set of fishes were intramuscularly injected with sterile saline suspension

Cumulative mortalities of fishes with respect to number of days after inoculation is shown in Table 4. The table shows that in all cases there is a steep rise in the number of mortalities after one after inoculation of pathogenic bacteria PF-02. The maximum number of death of fishes occurred during 4 to 7 days hours after inoculation. After 7 days, the death rate fell steeply and no further death in the fishes were recorded. In Cirrhinus mrigala the death rate of fishes injected with PF-02 was slightly varied in fishes' species inoculated. The percentage mortality was higher in Cirrhinus mrigala.

Table 4 relation of time and Mortalities of the Cirrhinus mrigala after injection with bacterial suspension of AH-04

Days after inoculation	*Number of fishes inoculated*	**PF-02**		**PF-14**
		Number of fishes dead	*% morality*	*Number of fishes dead*
1	20	0	0	0
2	20	3	15	3
3	20	8	40	7
4	20	11	55	9
5	20	13	65	11
6	20	14	70	12
7	20	15	75	13
8	20	16	80	14
9	20	16	80	15
10	20	16	80	15
11	20	16	80	15
12	20	16	80	15

Discussion:

In the present work, the serially diluted dose of different bacterial suspensions produced consistent trends of mortality. Isolation and identification of injected bacterial strains from external lesion, liver, and kidney of freshly dead fishes clearly indicate that the cause of death was associated with these bacterial strains. Various workers have observed changes in the liver and kidney of fishes infected by pathogenic strain of Pseudomonas fluorescens from liver, kidney and spleen of fish experimentally infected by the bacteria(Pal and Pradhan, 1995). Prasad et al., (1995).

The LD50 values of the two Pseudomonads (PFS01 and PFS07) in fishes were found to be 1 X 105 c.f.u., for all four isolates. Descriptions of the degree of virulence by previous workers (Mittal·et al, 1980; Santos et al, 1996) suggested that strains of Pseudomonas sp. showing LD50 less than 1X 106 could be classified as virulent, those with LD50 106 to 108 as weakly virulent and those with LD50 more than108 as nonvirulent. Accordingly PFS01 and PFS07 could be denoted as virulent. There was no significant difference between the virulence of these. The virulence was similar as suggested by its slightly higher LD50 value.

Aeromonads and Pseudomonads were found to experimentally induce ulcerative symptoms like skin lesion when injected intramuscularly to healthy Catla catla and Labeo rohita using at least 1X 105 c.f.u./ml (Lio-Po etal, 1992; Leung etal, 1995). Sahu et al., (1996) found that experimentally infected Pseudomonas fluorescens could produce lesion in the catfish, Clarias batrachus on 30th day and in Rohu (Labeo rohita) on the 10th day. However, the infection was limited to ulcer in the skin and muscle only. Histopathological changes in the internal organs were not as remarkable as it was in the skin and muscle. Pseudomonas sp., various other types of bacteria were also found to be associated with epizootic ulcerative syndrome. Kar et al., (1990) found E. coli and Pseudomonas aeruginosa to be associated with epizootic ulcerative syndrome affected fishes.

Ali and Tamuli (1991) isolated three types of bacteria from ulcers of four species of affected fishes and reinfection studies showed that Aeromonas sp. induced only mild infections, Vibrio sp. induced similar disease symptoms and Micrococcus sp. failed to induce any disease symptom.

Qureshi et al (1995) isolated nine types of bacteria from EUS affected fishes out of which Pseudomonads and Aeromonads were found to be highly pathogenic These previous findings show that there is little similarity in the type of bacteria isolated from epizootic ulcerative syndrome positive fishes though many of these bacteria are virulent and are able to induce epizootic ulcerative syndrome like lesion when injected to healthy fishes. AHS02 and AHS03 also belong to different genera and species though they are almost equally virulent. A possible explanation for this discrepancy in bacterial types may be that epizootic ulcerative syndrome is not caused by any single bacterium. It seems to be a complex disease caused by mixed infection.

Different species of Pseudomonas has been reported to be the causative agent of various fish diseases throughout the world. Pseudomonas anguilliseptica was identified as the etiological agent of red spot disease in Japan characterized by petechial haemorrhage in the mouth, opercula and ventral portion of the body of the fish (Nakai etal, 1985). P. anguilliseptica was also isolated from red spot disease of pond cultured eel, A. japonica in Taiwan (Kuo and Kou, 1978), from A. anguilla in Scotland (Stewart et al, 1983) and from salmonid fish in Finland (Wiklund and Bylund, 1990). Muroga and Nakajima (1981) reported artificial induction of red spot disease in Anguilla japonica with P. anguilliseptica and was able to induce the same clinical signs as those observed during disease outbreak by reinfection tests. Pseudomonas fluorescens has been reported to cause haemorrhagic septicemia in European eel, Anguilla vulgaris (Andre et al, 1970), pond cultured tilapia, Sarotherodon niloticus (Miyashita, 1984; Miyazaki etal, 1984), yellowtail Seriola quinqueradiata (Kusuda, 1980) and cyprinid fishes (Shiose etal, 1972). Sakai etal (1989) isolated P. fluorescens from diseased rainbow trout Onchorhinchus mykiss in Japan and found the bacteria to be pathogenic to rainbow trout and tilapia (S. niloticus). Saeed et al (1987) isolated P. putrefaciens from diseased rabbit fish, Siganus rivulatus in Red sea. Pal and Pal (1986b) reported induction of ulcer in A. testudineus by mixed culture of two bacteria, one fluorescent Pseudomonad and another, Micrococcus varians.

The fish diseases which involve Pseudomonas fluorescens includes motile Aeromonad septicemia, red spot disease of European eel, Anguilla anguilla (Schaperclaus, 1934), red

disease of Japanese eel, A. japonica (Hoshina, 1962) and red disease of carp, Cirrhinus mrigala (Egusa, 1978). Jo and Onishi (1980) isolated A. hydrophila from all diseased cultured ayu, Plecoglossus alfivelis characterized by exopthalamus and subcutaneous ulceration. Rahim et al., (1985) isolated A. hydrophila from the wounds of five species of fishes in Bangladesh. Okpokwasili and Okpokwasili (1994) found that Pseudomonas spp. and A. hydrophila isolated from brown patch disease of tilapia were more virulent to tilapia fingerlings when infected by a mixed culture than A. hydrophila or Pseudomonas spp. alone. Esteve et al., (1993) isolated Pseudomonas fluorescens and Aeromonas jandaei from diseased European eel (Anguilla anguilla) from an eel farm in Spain which caused ulcerous disease by intraperitoneal injection (LD50 dose: 1X105 to 1 X 107 c. f. u. / fish) and also by bath exposure to 107 to 108 c.f.u./ ml in healthy eels.

Several other workers (Chattopadhyay etal, 1990; McGaray etal, 1991; Torres et al1993; Cartwright et al1994.) also reported the association of mainly Aeromonas and occassionally Pseudomonas with epizootic ulcerative syndrome. Among Aeromonads, Karunasagar etal (1989) and McGaray etal (1991) had recovered Aeromonas hydrophila and Aeromonas sobria more often than other bacteria. Pal and Pradhan (1990) on the other hand found Aeromonas caviae and two other fluourescent Pseudomonads to be involved in EUS. This tends to indicate that Aeromonas sp. and Pseudomonas sp. are highly opportunistic pathogens which invade the fish once the skin barrier is breached. This however does not eliminate the fact that these bacteria are primary pathogens. In order to arrive at a conclusion on this aspect, a detailed study on the role of these bacteria in causing EUS is necessary.

This tends to indicate that Aeromonas sp. are highly opportunistic pathogens which invade the fish once the skin barrier is breached. This however does not eliminate the fact that these bacteria are primary pathogens. In order to arrive at a conclusion on this aspect, a detailed study on the role of these bacteria in causing epizootic ulcerative syndrome is necessary.

Reference

Ali, A. and K. Tamuli. 1991. Isolation of an etiological agent of epizootic ulcerative syndrome. Fish. Chimes. 11: 43-46.

Andre, P. G., D. A. Conroy, D. McGregor, R. J. Roberts and H. Young. 1970. Acute hemorrhagic scepticemia in captive European eel (Anguilla vulgaris} :A clinical pathological study. Vet. Rec. 90: 726-729.

Barde, R. D. (2021). Bacterial Diversity Associated With Labeo Rohita Isolated From Cultured And Natural Water Bodies. European Journal of Molecular & Clinical Medicine, 7(11), 2020.

Barde, R. D., Apastambh, A. R., & Baig, M. M. V. (2021). Association of Oomycetes Aphanomyces invadans with Freshwater fishes. Annals of the Romanian Society for Cell Biology, 25(6), 14118-14125.

Barde, R. D., Deshpande, M., Nagthane, N., Darak, O., & Baig, M. M. V. (2020). A Review of Saprolegnia Infection in Freshwater Fishes and Control of the Saprolegniosis. Sustainable Humanosphere, 16(1), 702-711.

Bergey's manual of Systematic Bacteriology 1986. Vol.II (Eds.A. Peter, A. Sneath and M. Elizabath sparbe). Williams and Wikins, Baltimore. pp. 156-170.

Cartwright, G. A., D. Chen, P. J. Hanna, N. Gudkovs and K. Tajima. 1994. lmmunodiagnosis of virulent strains of

Aeromonas hydrophila associated with epizootic ulcerative syndrome (EUS) using a monoclonal antibody. J. Fish Dis. 17: 123-133.

Chattopadhyay, U. K., D. Pal, M. S. Das, S. Das and R. N. Pal. 1990. Microbiological investigations into epizootic ulcerative syndrome in fishes. Proc. Natl. Workshop on Ulcerative Disease Syndrome in Fish. 6-7 March, 1990, Calcutta.

Cheesbrough, M. 1989. Medical laboratory manual for tropical countries, Microbiology, Vol.II, Ed. Tropical health technology, Butterworth and Co.Ltd.,Cambridge, pp. 216-252.

Darak, O. S., & Barde R.D., (2014). Association of bacterial pathogens with Labeo rohita in Marathwada region of Maharashtra. Int. J. Adv. Biotechnol. Res. l5, 777-783.

Darak, O. S., Baisthakur, P. O., & Barde, R. D. (2021). Antibiotic Resistance in Micrococcus SP Isolated from Freshwater Fish Farms Catla Catla. EFFLATOUNIA-Multidisciplinary Journal, 5(2).

Darak, O., & Barde, R. D. (2015). Pseudomonas fluorescens associated with bacterial disease in Catla catla in Marathwada region of Maharashtra. International Journal of Advanced Biotechnology and Research, 6(2), 189-195.

Egusa, S. 1978. Infectious diseases of fishes Koseisha-Koseikaku, Tokyo, Japan. pp.

Esteve, C., E. G. Biosca and C. Amaro. 1993. Virulence of Pseudomonas fluorescens and some other bacteria isolated from Euoropean eels, Anguilla anguilla reared in fresh water. Dis. Aquat. Org. 16: 15-20.

Hoshina, R. 1962. Studies on red disease of Japanese eel. J. Tokyo Univ. Fish. 6: 1-104.

Jo, Y. and K. Onishi. 1980. Pseudomonas fluorescens isolated from cultured Ayu (Plecoglossus altive/is). Fish Pathol. 15: 85-90.

Kar, D., R. G. Michael, S. Kamad and S. Changkija. 1990. Studies on fish epidemics from Assam. J. Inland Fish Assoc. 20: 73-75.

Karunasagar, 1., G. M. Rosalind, I. Karunasagar and Gopal Rao, K. 1989. Aeromonas hydrophila scepticemia of Indian major carps in some commercial fish farms of West Godavari district, Andhra Pradesh. Curr. Sci. 58: 1044-1045.

Kuo, S. K. and G. H. Kou. 1978. Pseudomonas anguilliseptica isolated from red spot disease of pond cultured eel Anguilla japonica. Rep. lnst. Fish. Bioi. Min. Econ. Aff. Nat. Taiwan University. 3: 19-23.

Kusuda, R. 1980. Pathogenic bacteria and diseases in marine fishes. Mari. Sci. Month. 126: 293-298.

Leung, K. Y., I. V. Yeap, T. J. Lam andY. M. Sin. 1995. Serum resistance as a good indicator for virulence in Pseudomonas fluorescens strains isolated from diseased fish in south-east Asia. J. Fish Dis. 18:511-518.

Lio-Po, G. D., L. J. Albright and E. V. Alapide-Tendencia. 1992. Pseudomonas fluorescens in the epizootic ulcerative syndrome (EUS) of snakehead (Ophiocephalus striatus) and catfish (Clarias batrachus): quantitative estimation in natural infection and experimental induction of dermomuscular necrotic lesion. In Diseases in Asian Aquaculture I. M. Shariff, R. P. Subasinghe, and J. R. Arthur (eds.) pp 431-436. Fish Health Section, Asian Fish. Soc. Manila.

McGarey, D. J., L. Milanesi, D. P. Foley, B. Reyes (Jr.), L. C. Frye and D. V. Lim. 1991. The role of motile aeromonads in the fish disease, ulcerative disease syndrome. Experientia. 47:441-444.

Mittal, K. R., G. Lalonde, D. Leblanc, G. Olivier and R. Lallier. 1980. Pseudomonas fluorescens in rainbow trout: relation between virulence and surface characteristics. Can. J. Microbial. 26: 1501-1503.

Miyashita, T. 1984. Pseudomonas fluorescens and Edwardsiella tarda isolated from diseased tilapia. Fish Pathol. 19: 45-50.

Miyazaki, T., S. S. Kubota and T. Miyashita. 1984. A histopathological study of Pseudomonas fluorescens infection in tilapia. Fish Pathol. 19: 161-166.

Muroga, K. and K. Nakajima. 1981. Red spot disease of cultured eels- methods for artificial infection. Fish Pathol. 15: 315-318.

Nakai, T., H. Hanada and K. Muroga. 1985. First record of Pseudomonas anguilliseptica infection in cultured ayu, Plecog/ossus a/five/is. Fish Pathol. 20 : 481-484.

Okpokwasili, G. C. and N. P. Okpokwasili. 1994. Virulence and drug resistant patterns of some bacteria associated with ubrown patch" disease of tilapia. J. Aqua. Trop. 9 : 223-233.

Pal, J. and B. C. Pal. 1986. Induction of tumors by bacterial culture. In Perspective in Cytology and Genetics, Vol 5. {G. K. Manna and U. Sinha, eds.). pp. 661-668. Indian Congress of Cytology and Genetics, New Delhi.

Pal, J. and K. Pradhan. 1990. Bacterial involvement in ulcerative condition of air breathing fish from India. J. Fish Bioi. 36: 833-839.

Pal, J. and K. Pradhan. 1995. Histopathology of liver, kidney and spleen of experimentally infected fish, Clarias batrachus. Fourth Asian Fisheries Forum, Abstracts, Oct. 16-20, 1995, Beijing, China. pp. 46.

Prasad, Y., T. A. Qureshi, S. A. Mastan and R. Chouhan 1995. Studies on the pathogenicity of Pseudomonas fluorescens isolated from EUS affectd fishes. Fourth Asian Fisheries Forum, Abstracts. October 16-20, 1995, Beijing, China. pp. 38.

Qureshi, T. A., Y. Prasad, S. A. Mastan and R. Chouhan. 1995. Bacteriological investigations on EUS affected fishes. Fourth Asian Fisheries Forum, Abstracts. October 16-20, 1995, Beijing, China. pp. 39.

Rahim, Z., K. M. S. Aziz, M. I. Haque and H. Saeed. 1985. Isolation of Pseudomonas fluorescens from the wounds of five species of brackishwater fish of Bangladesh. Bangladesh J. Zoo/. 13: 37-42.

Saeed, M. 0., M. M. Alamoudi and A. H. AI Harbi. 1987. A Pseudomonas associated with disease in cultured rabbit fish Siganus rivulatus in the red sea. Dis. Aquat. Org. 3: 177-180.

Sahu, B. B., Gayatri Murjani and S.C. Mukherjee. 1996. Aeromonas infection in freshwater fish and catfish. National workshop on fish and prawn disease epizootics and quarantine adoption on India. Oct. 9, 1996. Central Inland Capture Fisheries Research Institute, Barrackpore, India. pp 87-93.

Sakai, M., S. Atsuta and M. Kobayashi. 1989. Pseudomonas fluorescens isolated from diseased rainbow trout Onchorhinchus mykiss. Kitasato Arch. of Exp. Med. 62: 157-162.

Santos, Y. Bandin, I. and Toranzo, A. E. 1996. Immunological analysis of extracellular products and cell surface components of motile Aeromonas isolated from fish. J. Appl. Bacteriol. 81 : 585-593.

Schaperclaus, W. 1934. Untersuchungen uberdielseuchen in deutshen kinnerund kusten wasseren 1930-1933. Zeit. Fisch. 32: 191-217.

Shiose, J., H. Wakabayashi, M. Tominaga and S. Egusa. 1972. A report on a disease of cultured carp due to capsulated Pseudomonas. Fish Pathol. 9 : 79-83.

Stewart, D. J., K. Woldemarious, G. Dear and F. Mochaba. 1983. An outbreak of 'Sekiten-byo' among cultured European eels Anguilla anguilla L. in Scotland. J. Fish Dis. 6: 75-76.

Torres, J. L., K. Tajima and M. Shariff. 1993. Numerical taxonomy and virulence screening of Aeromonas spp. isolated from healthy and ulcerative disease syndrome-positive fishes. Asian Fish. Sci. 6: 11-22.

Wiklund, T. and G. Bylund. 1990. Pseudomonas anguilliseptica as a pathogen of salmonid fish in Finland. Dis. Aquat. Org. 8: 13-19.