Asian Fisheries Science **13**(2000): 105-115
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.2000.13.2.002

Recovery of Multiple Drug Resistant Pseudomonads Associated with an Ulcerative Condition in an Airbreathing Murrel, *Channa gachua* Bl.

N. NABI, M. JABEEN and A. HASNAIN*

Section of Genetics
Department of Zoology
Aligarh Muslim University
Aligarh-202 002, India

Abstract

Three species of *Pseudomonas* namely *P. aeruginosa*, *P. putida* and *P. stuzeri* were isolated from the sera, kidneys and livers of *Channa gachua* (Bloch) suffering from an ulcerative condition. Involvement of *P. stuzeri* in the ulcerative condition of fish is being reported for the first time. All the strains showed resistance to 11 out of the 15 drugs monitored in the present case, whereas *P. stuzeri* exhibited additional resistance to cotrimoxazole. Agarose gel electrophoresis against the control obtained by curing with sodium dodecyl sulfate revealed that all three species harboured plasmids which transformed susceptible *Escherichia coli* HB101 host. Transformation experiments demonstrated that except resistance to cotrimoxazole and ceftimaxazole all the others were plasmid-borne. Sensitivity to gentamycin, chloramphenicol and cefotaxime was also exhibited in each strain. Upon challenging the host using pure cultures of either strain, only symptoms of erythema and skin aberrations could be observed, while mixed culture of all the three induced moderate ulcers. On the basis of review of already published evidence, *C. gachua* may be considered as a host of exceptional endurance to ulcerative condition.

Introduction

A variety of pathogens are known to be associated with ulcerative conditions in fishes (Roberts 1989; Das and Das 1993). Their association to bacteria as secondary pathogens has often been reported, regardless of whether the predisposing or etiological factors were viral (Frerichs et al. 1993), mycotic (Roberts et al. 1993; Viswanath et al. 1997), environmental (Sneizko 1974; Das et al. 1994) or a combination of them (Ghittino 1972; Raghuvendra et al. 1995).

**Corresponding author*

Pseudomonads, which the present investigation deals with, belong to a significant fraction of bacterial flora generally recovered from fish lesions. For instance, Schaperclaus (1959) recovered *Pseudomonas flourescence* strains from carp intestine suffering from dropsy, whereas Meyer and Collar (1964) showed its occurrence in moribund *Ictalurus* sp. Bullock et al. (1965a) isolated this bacterium from diseased gold fish and demonstrated its virulence following intramuscular injections. The same group of workers recovered as many as 19 strains of *Pseudomonas* from sick freshwater fish species (Bullock et al. 1965b). Internal organs of afflicted Japanese eel and septicaemic ulcers of sea perch and grouper were also found to harbour pseudomonad flora (Chen and Kou 1987; Nash et al. 1986). Likewise, Antychowicz and Rogulska (1986) and Evenberg et al. (1986) reported a frequent recovery of *Pseudomonas f*rom erythrodermatitic ulcers of *Cyprinus carpio* or the ones experimentally induced in this fish by *Aeromonas salmonicida* infection. However, secondary occurrence of pseudomonads was found to be rather occasional in several culture and wild fish species of Southeast Asia (Boonyaratpalin 1989) as well as in *Channa striatus* (Subasinghe et al. 1990) which is a sister species of the host being investigated in the present study. Later on, even colonies of *P. aeruginosa* were detected on the surface and muscle lesions of several UDS afflicted fish species including the channids (Kar et al. 1990). Schaperclaus et al. (1991) pointed out the possibility of *P. putida* like organisms causing pathogenicity in eel and mirror carp which subsequently transformed into general septicaemia of inner organs. Pseudomonad flora represented as much as 80% of the total microbial load in the kidneys and livers of infected turbot inhabiting different farms (Toranzo et al. 1993). A parallel occurrence of pseudomonads along with some other flora in the habitat as well as in the internal organs of diseased ayu was shown by Natsugawa and Iida (1996) and in a different instance from hemorrhagic ascitis in the same fish by Wakabayashi et al. (1996).

In the above cited literature, drug resistance which is now a documented characteristic of pseudomonads (Aoki et al. 1980; Chen and Kou 1987; Padilla and Vasquez 1993) was not always monitored. During the investigation, specific attention has been paid to this aspect of three strains of *Pseudomonads* which were recovered from *Channa gachua* Bl. (Family:*Channidae*) suffering from an ulcerative condition. This is also the first report on the occurrence of *P. stuzeri* in an ulcerative fish. The investigations have also been extended to ascertain which of the observed resistances were actually mediated by plasmids. The extent of damage, which may be caused by the opportunistic infection of the recovered flora, has been demonstrated through symptomatic observations during infectivity experiments and a comparison with the external clinical signs of naturally afflicted host.

Materials and Methods

A total of 98 diseased specimens of fish *C. gachua* were collected from ponds and ditches receiving highly polluted industrial wastes from a chemi-

cal plant situated at Gajraula (U.P., India). About 30% of them exhibited early signs of disease such as erythema and white patches while some of them already manifested increased areas of endurance and small lesions on the dorsal surface of the body and caudal peduncle. Fish of healthy appearance, which served as the control, were captured from one of the distant and isolated localities in the same region as under laboratory conditions. They developed no disease during the course of investigations and several months thereafter.

In the laboratory, plastic tanks of 60 l capacity were used to keep the fish in batches of 10 fish per tank. Each tank was equipped with an aeration outfit. They were fed *ad libitum* with minced meat diet followed by a 12 hour change of water in tank. Every morning, three batches of diseased fish were given potassium permanganate bath (3 mg·l) for ten minutes and then transferred to a thoroughly cleansed water. This procedure was performed for 20 - 27 days depending on the survival of fish. Throughout the experiment, water temperature was maintained at 16 - 18ºC, which is the normal temperature of running water during peak winter, and routinely checked for D.O. and pH which were 5.2 - 5.6 mg·l and 7.2 - 7.4, respectively. Dissolved oxygen was estimated according to the modified version of Winkler technique (APHA 1989). Constant observations were made on the gradual progression of pathological symptoms among afflicted fish.

Standard aseptic techniques were used to obtain inocula from abscesses, ulcers, lesions, kidneys, livers, and sera of diseased fish. Mixed strains were purified following the streak plate method, while the strains were biotyped up to species level (Table 1).

Virulence tests of isolated strains were conducted as described by Schaperclaus et al. (1991), using four batches of healthy *C. gachua* (body wt. 0.1 - 0.2 kg). Each batch is composed five fish under the conditions outlined above. Subcutaneous injections of 0.2 ml (10^9 cells·ml) suspensions of mixed or individual culture of the desired isolates were given to these batches. For 20 days, observations were made on the condition of the fish for the appearance of already described symptoms of disease.

Antibiotic susceptibility of identified strains was determined by using susceptibility test discs (Span Diagnostic Ltd., India). The tests were performed as recommended by the supplier. Individual discs contained any one of the following drugs in indicated concentrations: tetracycline, 30 mcg; streptomycin, 10 mcg; gentamycin, 10 mcg; chloramphenicol, 30 mcg; erythromycin, 15 mcg; cotrimoxazole, 25 mcg; kanamycin, 30 mcg; ampicillin/ sulbactam, 20 mcg; olfloxacin, 5 mcg; amikacin, 30 mcg; cefotaxime, 30 mcg; piperacillin, 100 mcg; ciprofloxacin, 5 mcg; ceftizoxime, 30 mcg or pefloxacin, 10 mcg.

After screening for the presence of plasmids in purified *Pseudomonas* strains, transformation experiments were performed using any of the three isolated strains as a donor (Davis et al. 1986a). Plasmids from any of the donor strains were isolated following the standard “mini prep” method (Davis et al. 1986b). *Escherichia coli* strain HB101 with streptomycin resistance as the chromosomal marker was used as the host. The presence of plasmid in a

transformant was checked through electrophoresis in 1% agarose gel by direct screening of alkali lysate of culture obtained from the inoculum of individual colony. Transformants were also tested for each drug resistance already recorded for the donor strains. To produce plasmidless controls, a few curing experiments using sodium dodecyl sulfate were also performed on each strain.

Results

Three days after being taken to the laboratory, swollen white patches (Fig.1a) on the body of afflicted fish turned into red and pale lesions with

Table 1. Morphological, physiological and biochemical characteristics of three species of *Pseudomonas* involved in the ulcerative disease of *Channa gachua*.

Tests	*P. putida*	*P. stuzeri*	*P. aeruginosa*
Gram's staining	-	-	-
Form	Rod	Rod	Rod
Spores	-	-	-
Motility	+	+	+
Growth at			
5°C	+	-	-
42°C	-	+	+
50°C	-	-	-
65°C	-	-	-
Growth on NaCl%			
2.5%	-	+	-
7%	-	-	-
Growth at pH			
5.7	+	+	-
9	-	+	+
Growth on MacConkey agar	+	+	+
Citrate utilization	+	-	-
Starch hydrolysis	-	-	-
Casein hydrolysis	+	-	-
Urea hydrolysis	-	±	-
Indole	+	+	+
Methyl Red (MR)	-	+	+
Voges Proskauer (VP)	-	-	-
Nitrate reduction	-	+	-
H2S Production	-	-	-
Catalase	+	+	+
Oxidase	+	+	+
Gelatin lequifaction	-	-	-
Anaerobic growth	-	-	-
Utilization of carbohydrates (Acid production)			
Glucose	+	+	±
Xylose	+	+	-
Mannitol	-	+	+
Sucrose	-	-	-
Maltose	-	+	-
Lactose	-	-	-
Adonitol	-	-	-

white borders (Fig. 1b,c). Hemorrhages and irregular arrangement of scales were also noticed in the affected body parts (Fig. 1d). Lesions, specifically on the head, eye, and caudal peduncle gradually transformed into hemorrhagic and deep ulcers. The normal profile of snout that was maintained in the beginning altered following necrosis and resulted to severe damage in the dorsal surface of the head. At this stage, even eye lenses turned opaque, making the fish virtually blind (Fig. 1e). As a result of swelling and distortion on both lips concomitant with the initial distortion in the oral region and buccal cavity (Fig. 1f), fish stopped feeding and anomalous swimming that was noticed at the very onset of the disease became more pronounced. This condition was reached within one week of the initial symptom of the disease. Average mortality rate (50%) was high during the first 20 days when the most

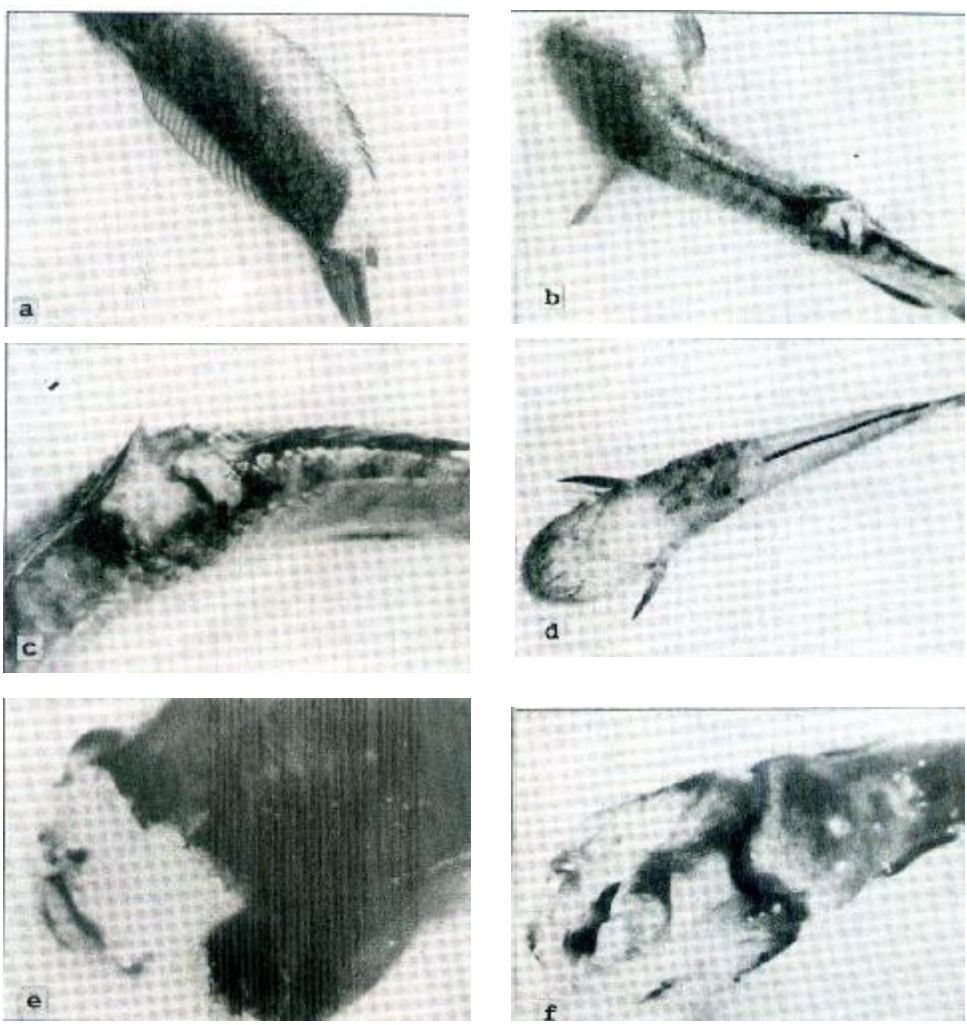

Fig.1. Photographs of infected *Channa gachua* showing: a) white patch at the base of caudal fin; b) initial stage of deep ulcer on dorsal fin but the normal external morphology of head; c) advanced stage of ulcer on the dorsal surface with a loss of fin continuity; d) ventral surface of fish showing normal features of the snout and mouth along with localized hemorrhage and irregular arrangement of scales; e) severely damaged head and highly distorted outline of snout; f) terminal stage of infection with high degree of distortion of mouth and snout.

serious symptoms of the disease characterized by severe damage to the eye, buccal region and head affecting even the maxilla and mandible were recorded. Continued mortality was recorded hereafter, but about 15% still survived another 1 - 2 weeks in spite of the continued starvation. Among the fish treated with potassium permanganate, only moderate cleansing effect on ulcers was observed and these fish survived 2 - 3 days longer than the untreated batches. Thus, the therapeutic value of potassium permanganate within the concentration used here is doubtful.

The results of identification of associated bacterial pathogen determined by standard physiological, morphological, and biochemical tests are shown in Table 1. All the strains were Gram negative, nonspore forming motile rods and were found to be positive to indole, oxidase and catalase tests. They were identified as *Pseudomonas* (Buchanan and Gibbons 1974) and further classified up to species level as being *P. aeruginosa*, *P. putida* and *P. stuzeri* (Doudoroff and Palleroni 1974). No other bacteria could be isolated from the inocula of sera, kidneys or livers of the diseased fish.

Infectivity experiments revealed that subcutaneous inoculation of *P. aeruginosa* in healthy *C. gachua* induced superficial ulcers leading to the formation of small lesions while, either *P. stuzeri* or *P. putida* alone produced erythema and skin distortion only. Although a mixed culture of these three strains induced deep ulcers, the advanced fatal symptoms observed in diseased fish could not be reproduced among the challenged individuals. Consequently, based on the dose administered in the present study and the observation period of two months, mortality did not exceed 20%.

The results of antibiotic susceptibility of isolated strains against 15 drugs are given in Table 2. The most remarkable feature of these strains is their resistance against 11 drugs. All the strains were highly sensitive to gentamycin while chloramphenicol and cefotaxime ranked second. Although *P. putida* and *P. aeruginosa* were sensitive to cotrimoxazole, *P. stuzeri* exhibited resistance against this drug. Resistance to pefloxacin, kanamycin, streptomycin, erythromycin, ampicillin/salbactam, olfloxacin, amikacin, piperacillin, ciprofloxacin, ceftizoxime and tetracycline were shared by all three strains.

Results of transformation experiments are given in Table 3, according to which resistance to cotrimoxazole and ceftizoxime appears to have chromosomal locations in *P. stuzeri*. Curing results were in agreement with the phenotypic specificity of each strain established on the basis of transformation. Agarose gel electrophoresis of alkali lysates of inoculum from individual colonies of transformants confirmed the presence of transforming plasmids against the control HB101 (Fig. 2).

Discussion

A review of literature revealed that certain fish species of Southeast Asia, such as *Wallago attu*, *Puntius* sp. and two sister species of genus *Channa* are relatively susceptible to ulcerative disease (Das and Das 1993; Mohan and

Shankar 1994; Thampuran et al. 1995), though clinically these conditions might not have all been the same. *Channa gachua*, which the present investigations are concerned with, belongs to this "high susceptibility" group. It however, differs from the rest of the group in showing a remarkable endurance to the disease as it survived beyond three weeks after severe symptoms of the disease were initially recorded making it a good model for prolonged pathological investigations. The efficiency of accessory airbreathing organ alone may not fully account for the prolonged survival observed in the present case, since *Heteropneustes fossilis*, *Clarias batrachus* (Pal and Pradhan 1990) as well as

Table 2. Drug susceptibility of bacterial strains isolated from diseased fish (*C. gachua).*

Drug tested	*P. aeruginosa*	*P. putida*	*P. stuzeri*
Kanamycin	R	R	R
Cotrimoxazole	H.S.	H.S.	R
Chloramphenicol	S	S	S
Erythromycin	R	R	R
Gentamycin	H.S.	H.S.	H.S.
Tetracycline	R	R	R
Streptomycin	R	R	R
Ampicillin/Sulbactam	R	R	R
Cefotaxime	S	S	S
Piperacillin	R	R	R
Ciprofloxacin	R	R	R
Ceftizoxime	R	R	R
Olfloxacin	R	R	R
Amikacin	R	R	R
Pefloxacin	R	R	R

R = Resistance
H.S. = Highly sensitive
S = Sensitive

Table 3. Drug susceptibility of transformants.

Drug tested	*P. aeruginosa*	*P. putida*	*P. stuzeri*
Kanamycin	R	R	R
Cotrimoxazole	S	H.S.	H.S.
Chloramphenicol	S	S	S
Erythromycin	R	R	R
Gentamycin	H.S.	H.S.	H.S.
Tetracycline	R	R	R
Streptomycin	R	R	R
Ampicillin/Sulbactam	R	R	R
Cefotaxime	S	S	S
Piperacillin	R	R	R
Ciprofloxacin	R	R	R
Ceftizoxime	R	R	S
Olfloxacin	R	R	R
Amikacin	R	R	R
Pefloxacin	R	R	R

R = Resistance
H.S. = Highly sensitive
S = Sensitive

some sister species of genus *Channa* (Llobrere and Gacutan 1987; Thampuran et al. 1995) which were also equipped with such organs, did not match the endurance shown by *C. gachua*.

As far as progress of the disease is concerned, there exists a clear difference in the severity of the symptoms between the challenged, where lesions are restricted only to the skin, and naturally affected fish. This is in agreement with the results on a sister species *C. punctatus*, where a mixed inoculum of pseudomonads even in association with *Aeromonas* sp. failed to produce severe symptoms (Pradhan and Pal 1990), which indicated that isolates excluded the primary etiological agent and were devoid of environmental stress of the original habitat. Among others, the last mentioned (environmental) factor is one of the most apparent and remarkable differences between the respective habitats of the sick lot and the challenged specimen. The diseased fish, unlike the healthy ones (which were challenged in the laboratory) inhabited a visibly polluted ambient. The limited water analysis that was conducted showed it to be turbid, having an alkaline pH (8.6) besides being low in oxygen content (2.5 ppm). The role of such conditions in promoting the disease by weakening the dermal tissue had already been emphasized (Virgona 1992; Lilley et al. 1991). Pseudomonads, thus, might have colonized the lesions produced on these spots of *C. gachua* as secondary opportunistic pathogens. Whereas, two other species of *Pseudomonas*, namely: *P. aeruginosa* and *P. putida* have already been recorded in other instances (Bullock et al. 1965b; Lallier et al. 1981; Wong and Leong 1987), association of *P. stuzeri* as a secondary opportunistic pathogen in fish is being reported here for the first time.

Of direct relevance to the host pathogen's interaction and its correlationship with the environment is the remarkable multiplicity of drug resistances borne by three pseudomonads which flourish in the ulcerative

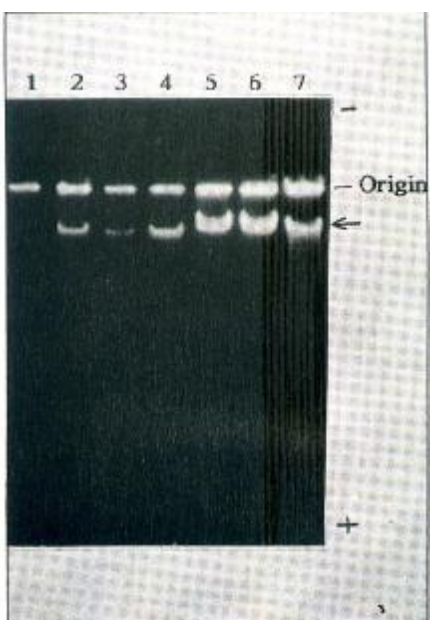

Fig. 2. Electrophoretic patterns of alkali lysates of cell pellets of three strains of Pseudomonas recovered from diseased *Channa gachua* and their corresponding transformants in 1% agarose gels. Arrow indicates the position of plasmid bands. From left to right, the lanes show: 1) control (plasmid-free *Escherichia coli* HB101); 2-4) transformants of *Pseudomonas aeruginosa*, *P. putia* and *P. stuzeri*, respectively; 5-7) donor *Pseudomonas* strains in the same order. Flourescence at the origin indicates the retained genomic DNA.

region of *C. gachua* (Table 2). As revealed by *in vitro* transformation experiments, of the 15 drug resistances monitored here, 11 were plasmid-borne in *P. aeruginosa* and *P. putida*; whereas in *P. stuzeri* only 10 belonged to this category. Two exceptions, that is, the resistances to cotrimoxazole and ceftizoxime apparently have chromosomal locations. Though the occurrence of pseudomonads in the ambient as well as in the host may be an established possibility in some cases (Aoki et al. 1980), they commonly inhabit both water and soil (Sonnenwirth 1980; Sandaa et al. 1992). Due to nutritional versatility, a better survival of *Pseudomonas*-like bacteria from polluted sites, as compared to unpolluted ones, had been recorded by Burton et al. (1982). In any case, plasmids from this genus can also transform a variety of bacteria under natural conditions (Davis 1980; Genthner et al. 1992; Padilla and Vasquez 1993) while a high frequency of transformation can be achieved in nonconjugative fish pathogenic strains, if conjugative helper plasmids from other sources are available (Hayashi et al. 1982). *Pseudomonas* with large plasmid-loads have been found to show high frequency of transformation along with a good survival rate in tropical waters (Cruz-Cruz et al. 1988). The evidence on the spatial spread of a variety of resistances by plasmids of *Pseudomonas* is, therefore convincing and chromosomal resistance as reported here adds to this concern.

Acknowledgment

We express our gratitude to the Chairman, Department of Zoology for providing the essential laboratory facilities. The help of IMTECH (Institute of Microbial Technology, Chandigarh) in identifying bacterial isolates is also acknowledged. This work was supported by the UGC (GATE) Scholarship received by N. Nabi.

References

APHA. 1989. Standard methods for examination of water and waste water. 16th ed. American Public Health Association, Washington D.C., 1134 pp.

Antychowicz, J. and A. Rogulska. 1986. Preliminary investigations on the bacterial flora of the skin and erythrodermatitic ulcers of carp. Bull. Vet. Inst. Pulawy. 28-29:42-45.

Aoki, T., Y. Jo and S. Egusa. 1980. Frequent occurrence of drug resistance bacteria in ayu (*Plecoglossus altivelis*) culture. Fish Path., Tokyo, 15:1-6.

Boonyaratpalin, S. 1989. Bacterial pathogens involved in epizootic ulcerative syndrome of fish in Southeast Asia. J. Aqua. Anim. Health, 1:272-276.

Buchanan, R.E. and N.E. Gibbons. 1974. Bergey's Manual of Determinative Bacteriology. 8th Edition. Williams and Wilkins. Baltimore, Maryland.

Bullock, G.L. 1965a. Characteristic and pathogenicity of a capsulated *Pseudomonas* isolated from gold fish. Appl. Microbiol., 13:89-92.

Bullock, G.L., S.F. Sneiszko and C.E. Durban. 1965b. Characteristics and identifications of oxidative pseudomonads isolated from diseased fish. J. Gen. Microbiol., 38:1-7.

Burton, N.F., M.J. Day and A.T. Bull. 1982. Distribution of Bacterial Plasmids in clean and polluted sites in a South Wales river. Appl. Environ. Microbiol. 44:1026-1029.

Chen, H.Y. and G.H. Kou. 1987. Studies on bacterial drug resistance in aquaculture I Drug resistance of bacteria in pond reared eels (*Anguilla japonica*). The Memoir of Bacteriology and Immunology in Fish Disease. 10: 12-24.

Cruz-Cruz, N.E., G.A. Toranzos, D.G. Ahearn, and T.C. Hazen. 1988. In situ survival of plasmid bearing and plasmidless *Pseudomonas aeruginosa* in pristine tropical waters. Appl. Environ. Microbiol. 54:2574-2577.

Das, M.K. and R.K. Das. 1993. A review of the fish disease epizootic ulcerative syndrome in India. Environ. Ecol. 11:134-145.

Das, M.K., R.K. Das, S.P. Ghosh and S. Bhowmick. 1994. Fish disease, its relation with environmental factors in a sewage fed wetland. J. Inland Fish. Soc. India. 26:100-105.

Davis, L.G., M.D. Milner and J.F. Battey. 1986a. Basic methods in molecular biology. Elsevier Science Publishing Co. Inc., New York. 90-92 pp.

Davis, L.G., M.D. Milner and J.F. Battey. 1986b. Basic methods in molecular biology. Elsevier Science Publishing Co., Inc. New York. 102-104 pp.

Davis, B.D. 1980. Gene transfer in bacteria. In: Microbiology, 3rd edn. (eds. B.D. Davis, R. Dulbeco, H.N. Eisen and H.S. Ginsberg), 138-150 pp, Harper and Row Publishers, New York.

Duodoroff, M. and N.J. Palleroni. 1974. Genus I. *Pseudonomas.* N: Bergy's Manual of Determining Bacteriology (eds. R.E. Buchanan and N.E. Gibbons), Williams and Wilkins, Baltimore, 217-243 pp.

Evenberg, D., P. de-Graaf, W. Fleuren, van-Muiswinkel 1986. Blood changes in carp (*Cyprinus carpio*) induced by ulcerative *Aeromonas salmonicida* infections. Vet. Immunol. Immunopathol. 12: 321-330.

Frerichs, G.N., S.D. Millar and S. Chinabut. 1993. Clinical response of snakeheads (*Ophicephalus striatus*) to experimental infection with snakehead fish rhabdovirus and snakehead cell line retrovirus. Aquaculture, 116:297-301.

Genthner, F.J., R.P. Campbell, P.H. Pritchard. 1992. Use of a novel plasmid to monitor the fate of a genetically engineered *Pseudomonas putida* strain. Mol. Ecol. 1:137-143.

Ghittino, P. 1972. The principal aspects of bacterial fish disease in Italy. Symposia of The Zoological Society of London, 30:25-38.

Hayashi, F., Y. Araki, K. Harada, M. Inoue and S. Mitsuhashi. 1982. Epidemiological studies of drug resistant strains in cultured fish and water. Bull. Jap. Soc. Sci. Fish. 48:1121-1127.

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

Lallier, R., K.R. Mittal, D. Leblanc, G. Lalonde and G. Olivier. 1981. Rapid methods for the differentiation of virulent and nonvirulent *Aeromonas hydrophila* strains. Dev. Biol. Standards 49:119-123.

Lilley, J.H., M.J. Phillips and K. Toguthai. 1991. A review of epizootic ulcerative syndrome (EUS) in Asia. Aquatic Animal Health Research Institute ND network of Aquaculture Centers in Asia-Pacific, Bangkok, 73pp.

Llobrera, A.T. and R. Q. Gacutan. 1987. *Aeromonas hydrophila* associated with ulcerative disease epizootic in Laguna de Bay, Philippines. Aquaculture 67:273-278.

Meyer, F.P. and J.D. Collar. 1964. Description and treatment of *Pseudomonas* infection in sohiter catfish. Appl. Microbiol. 25:155-156.

Mohan, C.V. and K.M. Shankar. 1994. Epidemiological analysis of epizootic ulcerative syndrome of fresh and brackish water fishes of Karnataka, India. Curr. Sci. 66:656-658.

Nash, G., I. G. Anderson, M. N. Shariff and N. Shamsuddin. 1986. Bacteriosis associated with the epizootic in the giant perch, *Lates calcarifer* and the estuarine grouper, *Epinephelus tauvina,* cage cultured in Malaysia. International Colloquium on Pathology in Marine Aquaculture. Oporto, Portugal, cited in Aquaculture. 1987. 67:105-111.

Natsugawa, T. and Y. Iida. 1996. *Pseudomonas* sp. isolated from diseased ayu *Plecoglossus altivelis.* Fish Path., 31:221-227.

Padilla, C. and C. Vasquez. 1993. Plasmid mediated antibiotic resistance in *Pseudomonas aeruginosa* from well water sediments and their transformation into *Escherichia coli.* Lett. Appl. Microbiol. 16:17-20.

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

Pradhan, K. and J. Pal. 1990. Experimental induction of ulcer in the fish *Channa punctatus* by bacteria. Environ. Ecol. 8:812-815.

Raghuvendra, Pai., I. Karunasagar, H.P.C. Shethy and I. Karunasagar. 1995. The effect of some stress factors on infection of fish by *Aeromonas hydrophila.* J. Aqua.Trop.10: 29-35.

Roberts, R.J. 1989. Fish Path., 2nd ed. Balliere Tindall, London. 318 pp.

Roberts, R.J., L.G. Willoughby and S. Chinabut. 1993. Mycotic aspects of epizootic ulcerative syndrome (EUS) of Asian fishes. J. Fish Disease. 16:169-183.

Sandaa, R.A., Torsvik, V.L., and J. Goksoyr. 1992. Transferable drug resistance in bacteria from fish-farm sediments. Can. J. Microbiol. 38:1061-1065.

Saye, D. J., O. Ogunseitan, G. S. Sayler and R. V. Miller. 1987. Potential for transduction of plasmids in a natural freshwater environment. Effect of plasmids donor concentration and a natural microbial community on transduction in *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 83:987-995.

Schaperclaus, W. 1959. *Pseudomonas flourescence* strains in carp intestine. Dt. Fishcwist. Ztg. 6:176-179.

Schaperclaus, W., Kulow, H. and K. Schreckenbach. 1991. Fish Diseases.1. Oxonian Press New Delhi, 378-379 pp.

Schaperclaus, W., H. Kulow and K. Schreckenbach 1991. Fish Diseases.3. Oxonian Press Pvt. Ltd. New Delhi. 500-501 pp.

Sniezko, S.F. 1974. The effect of environmental stress on outbreaks of infectious diseases of fishes. J. Fish Biol. 6: 197-208.

Sonnenwirth, A.C. 1980. Pseudomonads and other non fermenting bacilli. In: Microbiology, 3rd edn. (eds. B.D. Davis, R. Dulbecco, H.N. Eisen and H.S. Ginsberg), 674 pp, Harper and Row Publishers, New York.

Subasinghe, R.P., Jayasinghe, L.P., Balasurya, K.S.W. and M. Kulathilake. 1990. Preliminary investigations into the bacterial and fungal pathogens associated with the ulcerative fish disease syndrome in Sri Lanka. Proceedings of the second Asian Fisheries Forum., Tokyo, Japan. (eds. R. Hirano and I. Hanyu). 655-657 pp.

Thampuran, N., P.K. Surendran, M.K. Mukundan and K. Gopakumar. 1995. Bacteriological studies on fish affected by epizootic ulcerative syndrome (EUS) in Kerala, India. Asian Fish. Sci. 8:103-111.

Toranzo, A.E., B. Novoe, J.L. Romalde, S. Nunez, S. Deversa, E. Marino, R. Silva,E. Martinez,Figueras, J.L. Barja 1993. Microflora associated with healthy and diseased turbot (*Scophthalmus maximus*) from the farms in northeast Spain. Aquaculture 114:189-202.

Virgona, J. L. 1992. Environmental factors influencing the prevalence of a cutaneous ulcerative disease (red spot) in the sea mullet, *Mugil cephalus* L., in the Clarence river, New South Wales, Australia. J. Fish Diseases 15:363-378.

Viswanath, T. S., C.V. Mohan and K. M. Shankar 1997. Clinical and histopathological characterisation of different types of lesions associated with epizootic ulcerative syndrome (EUS). J. of Aqua.Trop. 12:35-42.

Viswanath, T.S., C.V. Mohan and K.M. Shankar 1998. Epizootic ulcerative syndrome (EUS) associated with a fungal pathogen in Indian fishes histopathology- a cause for invasiveness. Aquaculture 165:1-9.

Wakabayashi, H., Sawada, K., Ninomiya, K. and E. Nishimori. 1996. Bacterial haemorrhagic ascitis of ayu caused by Pseudomonas sp. J. Fish Disease. 31:239-240.

Wong, S.Y. and T.S. Leong. 1987. Current fish disease problems in Malaysia. In: Fish Quarantine and Fish Diseases in South and Southeast Asia: 1986 update (ed. J. R. Arthur) Special Publication No. 1, Asian Fisheries Society, Manila., 12-21 pp.

Manuscript recieved 18 June 1999; Accepted 14 January 2000.