PHOTOBACTERIOSIS IN SOME WILD AND CULTURED FRESHWATER FISHES IN EGYPT

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Abstract

Two hundred and seventy wild and cultured fishes (*Mugil cephalus*, *Mugil capito* and *Nile tilapia)* were collected from Alexandria, and investigated for the isolation of *Photobacterium*. The recovered bacteria were studied for the virulence, pathogenicity and antimicrobial sensitivity. The pathogenesis of the most virulent isolate was done experimentally through pathological investigations. Monitoring of the water quality was also carried out. Three isolates (ph1, ph2 and ph3) of *Photobacterium damselae subsp. damselae* were obtained from *Mugil cephalus*, *Mugil capito* and *Nile tilapia,* respectively. The first two recovered isolates caused 20% mortality among *Oreochromis niloticus* and *Cyprinus carpio*, while the third one (ph3) caused 40% and 30% mortalities among the two fish species, respectively. The infection by these bacteria was accompanied by increase in the unionized ammonia (0.08 mg/L), decrease in the dissolved oxygen (1.8 mg/L) and high pH value (9.8). The experimentally infected fishes showed skin darkening and hemorrhaging of the caudal fin and operculum. Internally, whitish pin-sized nodules were seen in the liver, spleen and kidneys. Histopathologically, the *Photobacterium,* during the acute phase of the disease, induced degenerative changes in the parenchymatous organs of the infected fishes with marked brain lesions and hematopoietic involvement, while in the chronic stage, granuloma formation and focal necrosis in the internal organs was evident. The three recorded isolates of the *Photobacterium* were sensitive to erythromycin, streptomycin and ampicillin, but totally resistant to chloramphenicol, oxytetracycline, oxalinic acid, kanamycin and ciprofloxacin. On the other hand, Ph1 isolate was also sensitive to furazolidone while Ph3 was sensitive to neomycin. We were able to isolate three isolates of *Photobacterium damselae subspecies damselae,* which varied in virulence and antimicrobial sensitivity, from the wild and cultured freshwater fishes at Alexandria, Egypt as a new recorded pathogen in this environment.

Key words: *Photobacterium,* antimicrobial sensitivity, *Oreochromis niloticus, Mugil cephalus*, *Mugil capito, Cyprinus carpio, histopathology, Alexandria.*

INTRODUCTION

Photobacterium damselae was originally described as a new pathogenic *Vibrio species* causing ulcers in *Chromis punctipinnis* (Love et al. 1981). Since then, reports on the pathogenicity of the species for several important cultured fish (yellowtail,

turbot, rainbow trout, sea bream, sea bass), other fish (shark) and mammals (dolphin, humans) have increased exponentially. The taxonomic status of *P. damselae* within the family Vibrionaceae has changed repeatedly. After its original description as *Vibrio damselae*, it was reclassified as a member of the genus Listonella, along with the fish pathogen *Listonella anguillara* (McDonell and Colwell 1985). It was subsequently transferred to the genus *Photobacterium* on the basis of phenotypic data (Smith *et al.* 1991), and further support was obtained from the phylogenetic analysis carried by Ruimy *et al.* (1994).

In a later study, the fish pathogen *Pasteurella piscicida*, which causes pasteurellosis in several fish species, was found to be a member of *Photobacterium damselae* according to phylogenetic analysis of 16S rDNA sequences and DNA relatedness (Gauthier *et al*. 1995). However, important phenotypic differences which existed between the two fish pathogens motivated their retention as separate subspecies: *P. damselae subsp. damselae* and *P. damselae subsp. piscicida*.

On the other hand, *Photobacterium damselae* subsp. *piscicida* (formerly *Pasteurella piscicida*) is the aetiological agent of fish pasteurellosis. This disease was first observed in the USA in populations of wild striped bass (*Moronesaxatilis*) and white perch (*Morone americanus*) (Snieszko *et al.*, 1964). In 1969, the pathogen became a serious problem in Japan, causing great economic losses in cultured yellowtail (Kusuda & Yamaoka, 1972). In 1990, *Pasteurella piscicida*, for the first time, became a threat to the Southern European fish farm industry. In several countries of the Mediterranean area, including France (Baudin Laurencin *et al.*, 1991), Italy (Ceschia *et al.*, 1991), Spain (Toranzo *et al.*, 1991), Greece (Bakopoulos *et al.*, 1995), Portugal (Baptista *et al.*, 1996), Turkey (Candan *et al.*, 1996), Malta (Bakopoulos *et al.*, 1997), Israel (Bakopoulos *et al.*, 1997b) and Croatia (Oraic *et al.*, 1998), the pathogen was responsible for severe outbreaks of pasteurellosis in cultured populations of sea bass(*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). The taxonomic position of the pathogen has been controversial. The organism was first placed in the genus *Pasteurella* and described as *Pasteurella piscicida* (Janssen & Surgalla, 1968), although the bacterium was clearly distinguishable from all other species within this genus by a number of morphological and biochemical properties. In 1995, the pathogen was classified in the genus *Photobacterium* as a subspecies of *Photobacterium damselae* on the basis of rRNA sequence and DNA±DNA hybridization data (Gauthier *et al.*, 1995).

Although, typing of *P. damselae subsp. damselae* has received less attention than *P. damselae subsp. piscicida*. However, data reported for the former subspecies indicated a greater heterogeneity, both at phenotypic and genotypic levels (Pedersen *et al.* 1997, Thyssen et al. 2000). As a general observation, the *Photobacterium spp.* induced significant losses in the farmed yellow tail (Snieszko *et al.*, 1964 and Egusa, 1983). Photobacteria were also reported as a fish pathogen in Great Britain (Ajmal and Hobbs, 1967) and Norway (Hostein and Bullock, 1976). The *Photobacteria spp.* was isolated in different occasions from diseased and apparently healthy fishes (Plumb, 1994). Nevertheless, this ubiquitous microorganism was not reported to induce diseases among freshwater fishes, Matsusata (1975) found that the disease incidence in yellowtail was high during rainy season where the water temperature is optimum (25 $^{\circ}$C) and the salinity dropped below 30 ppt.

The present study aimed to survey and isolates the *Photobacterium* from freshwater fish species (*Mugil cephalus, M. capito* and *Nile tilapia)*. In addition to, investigating its virulence and pathogenic effect besides their sensitivity to some selected antimicrobials.

MATERIALS AND METHODS

1. Sampling

Swabs samples from the kidney, spleen, liver, heart, gills and skin of 270 wild and cultured fishes (80 *Mugil Cephalus*, 80 *Mugil Capito* and 110 *Nile tilapia)* were collected during 2007 within a survey in the West region of Alexandria Governorates (El-Nasryia, El-Marutia and El-Nabouria canals) of Egypt as a part of a National Research Project. Thirty sex water samples (1 liter each) were collected (1 sample/ locality/ month) in sterile bottle and delivered to the laboratory, without delay, for chemical examinations

2. Bacterial isolation and identification

The collected swabs were smeared separately onto plate of Brain heart infusion agar (BHIA) to which 0.5 to 3% NaCl was added. The inoculated plates were incubated at 25 $^{\circ}$C for 2-5 days. The grown bacteria were then sub-cultured on thiosulphate citrate bile salts-sucrose agar (TCBS-1) and examined morphologically, microscopically and their biochemical characteristics were tested by both traditional biochemical methods using the schedule of Whitman (2004) and through using API-20E strips (Paster Inst. France) (Table, 1).

3. Water analysis

The PH and dissolved oxygen (DO) of the collected water samples were analyzed using a PH (WTW) and an oxygen meter (Oxy Guard Handy Gamma portable dissolved oxygen meter, Techno lab. Marketing Pty. Ltd, Tasmania). The un-ionized ammonia was calculated using the spectrophotometric method described by Steele (2001).

4. Virulence of the isolated strains:

It was done through the inoculation of 0.1 ml of the culture suspension (10^7 CFU/ml) from each of the recovered isolates. ph1 isolate (that isolated from *Mugil Cephalus*), ph2 isolate (that isolated from *Mugil Capito*) and ph3 isolate (that isolated from *Nile tilapia*) to 20 fish (10 *Oreochromis niloticus* and 10 *Cyprinus carpio*).

5. Determination of half lethal concentration dose (LD$_{50}$) for ph3 isolate

Based on the results of the virulence experiment, the ph3 isolate was chosen for this experiment as it was of higher virulence than other isolates. The virulence of ph3 was conducted by intramuscular inoculation of 0.1 ml from each bacterial dilution (10^{-1}-10^{-8}) into ten fish from each of *Oreochromis niloticus* and *Cyprinus carpio*. Mortalities were recorded for seven days after the inoculation and the half lethal dose was calculated by the Reed and Muench method (1938) (Table, 3).

6. Experimental infection

Eighty *O. niloticus* were used in this experiment and divided into four equal groups (20 fish/group). The first three groups served as replicates and were inoculated with the most pathogenic isolate of the photobacteria (ph3 isolate). The fourth group served as a control and was inoculated intraperitoneally (I/P) with normal sterile saline (0.2 ml / fish). The infection to the first three groups was carried out by I/P inoculation of 0.2 ml culture suspension (10^5 CFU / ml). All infected and control fish were kept under observation for four weeks. Re-isolation of the inoculated bacteria was done from the freshly dead fish.

7. Sensitivity of isolated bacteria to selected antimicrobial

The resistance of the isolated *Photobacterium* was determined by disk diffusion on The Mueller–Hinton agar (Difco.). Eleven antimicrobial agents were selected to represent different classes of antimicrobials. Based on the distributions of the inhibitory zone diameters and, where available, recommendations from the Clinical and laboratory Standards Institute (formerly National Committee for Clinical

Laboratory Standards) (CLSI/NCCLS, 2005), break point values were used to separate the sensitive isolates from the resistant.

8. Histopathological examination

Specimens from the brain, liver, spleen, kidney and intestine of experimentally infected fishes were collected after postmortem examination and fixed in neutral buffered formalin 10%. Paraffin blocks were prepared after routine processing of the specimens. Then, sectioned (5 μ m) and stained with hematoxylin and eosin (H & E) according to Drury and Wallington (1980).

RESULTS

The infected fishes (*Mugil cephalus*, *Mugil capito* and *Nile tilapia),* by *Photobacterium,* showed sluggish movement and darkness of the skin. The internal organs of diseased fishes appeared swollen specially the kidney, spleen and liver and there was an accumulation of bloody fluid in the abdominal cavity.

The cultured bacteria appeared convex, viscous, regular and opaque to translucent colonies. Other colonies, of shiny-grey-yellow coloration and 1-2 mm diameter, were developed after 72 h of the incubation. The isolated bacteria were Gram negative rods with bipolarity. The biochemical characteristics of the isolates are summarized in Table (1). Fifty isolates of *Photobacterium damselae subsp. damselae* were recovered from the investigated organs of the 270 investigated freshwater fishes, 12 isolates obtained from 80 *M. cephalus* (ph1), 10 isolates obtained from 80 *M. capito (*ph2) and 28 isolates recovered from 110 *Nile tilapia* (ph3) (Table 2).

The analysis of water revealed that the un-ionized ammonia was ranged between 0.06 to 0.08 mg/L along the course of the study. Also, during the same period, the temperature ranged between 14 to 22 ^{o}C, PH ranged between 8.7 to 9.8 while the dissolved oxygen ranged between 1.8 to 2.1 ppm.

The virulence experiment was done using the three isolates of the *Photobacterium* that obtained from the three investigated fishes (Ph1, Ph2 and Ph3). The Ph3 was highly virulent than other two isolates (Ph1, Ph2). The Ph3 induced 40% and 30% mortalities among *O. niloticus* and *Cyprinus carpio,* respectively, while the Ph1 and Ph2 induced 20% mortalities in both species.

Table 1. Biochemistry profile of *Photobacterium damselae subsp. damselae***.**

Test	Result	Test	Result
Oxidative/fermentative	+ / +	Adonitol	-
Cytochrome oxidase	+	Amygdalin	-
Catalase	+	Arabinose	-
Citrate, Simmons	-	Caprate	-
Gelatinase	-	Cellobiose	V
SIM	-	Dextrose	+
Sulphide	-	Dulcitol	-
Indole	-	Fructose	+
Motility *	+	Galactose	+
VP	+	Gluconate	+
H2S	-	Glucose	+
Indole (Peptone H_2O)	-	Glycerol	V
Nitrate reduction	V	Inositol	-
Tryptophanase	-	Lactose	-
Arginine decarboxylase	+	Malate	-
Lysine decarboxylase	+	Maltose	V
Ornithine decarboxylase	-	Mannitol	-
Esculin hydrolysis	-	Mannose	+
Beta-galactosidase	-	Melibiose	-
N-Acetyl-D-glucosamine	-	Raffinose	-
Phenyl-acetate	-	Rhmanose	-
Triple sugar iron	K / A	Salicin	-
Urease	-	Sorbitol	-
0/129 disk	+	Sucrose	V
TDA	-	Trehhalose	V
Adipate	-	Xylose	V

+ strains are positive, - strains are negative, v strain are variable.

- Motile by one or more un-sheated polar flagella.

Table 2. Incidence of *Photobacterium damselae subsp. damselae* isolated from different organs of Freshwater fish.

Organs \ Strains	Kidney	Spleen	Liver	Heart	Gills	Skin	Total
Ph 1	3	5	2	1	1	0	12
Ph 2	2	3	3	1	1	0	10
Ph 3	13	7	4	2	2	0	28
Total	18	15	9	4	4	0	50

Ph 1 strain isolated from M. Cephalus, Ph 2 strain isolated from M. Capito, Ph 3 strain isolated from O. niloticus.

The LD_{50} of the Ph3 isolate (Table, 3) was $10^{-4.5}$ in *O. niloticus* and $10^{-2.5}$ in *Cyprinus carpio* based of the result of the following equation,

LD_{50} in *O. niloticus*: Proportionate distance (P.D.) = $\dfrac{60-50}{60-40}$ = 0.5

$LD_{50} = 4 + 0.5 = 4.5 = 10^{-4.5}$ $LD_{50} = 10^{-4.5}$

LD_{50} in *Cyprinus carpio*: Proportionate distance (P.D.) = $\dfrac{60-50}{60-40}$ = 0.5

$LD_{50} = 2 + 0.5 = 2.5 = 10^{-2.5}$ $LD_{50} = 10^{-2.5}$

Table 3. LD_{50} of Ph3 *Photobaterium damselae subsp. damselae** in *Oreochromis niloticus* and *Cyprinus carpio*.

Dilution of bacteria	Mortality/No. of tested O. niloticus	Mortality/ No. of tested Cyprinus carpio
10^{-1}	8 / 10	6 / 10
10^{-2}	7 / 10	6 / 10
10^{-3}	7 / 10	5 / 10
10^{-4}	6 / 10	4 / 10
10^{-5}	5 / 10	4 / 10
10^{-6}	4 / 10	4 / 10
10^{-7}	4 / 10	3 / 10
10^{-8}	3 / 10	0 / 10
Control	0 / 10	0 / 10

Ph3 isolated strain from O. niloticus, Mortality recorded for seven days after injection.

The antimicrobial sensitivity test revealed that, three isolates of the *Photobacterium* were sensitive to erythromyein, streptomycin and ampicillin, but totally resistant to chloramphenicol, oxytetracycline, oxalinic acid, kanamycin and ciprofloxacin. The Ph1 isolate was also sensitive to furazolidone while Ph3 was sensitive to neomycin (Table 4).

Table 4. Antibiogram of the three *Photobacterium* isolates.

Antibiotic	Ph 1	Ph2	Ph3
Chloramphenicol (50 μg)	R	R	R
Erythromyein (50 μg)	s	s	s
Oxytetracycline (50 μg)	R	R	R
Oxalinic acid (50 μg)	R	R	R
Neomycin (30 μg)	R	R	s
Streptomycin (25 μg)	s	s	s
Sulphodimidine (500 μg)	s	R	R
Ampicillin (30 μg)	s	s	s
Furazolidone (100 μg)	s	R	R
Kanamycin (30 μg)	R	R	R
Ciprofloxacin (30 μg)	R	R	R

S = Highly sensitive   s = moderately sensitive   R = Resistant

The experimentally inoculated fish, by the Ph3 isolate of the *Photobacterium damselae subsp. damselae* showed skin darkening and mild hemorrhages at the

caudal fin and operculum, in addition to scattered hemorrhages on the dorsal musculature. The gills were congested in some cases and anemic in others. The postmortem lesions were enlargement of the kidney and presence of multiple whitish-pin- sized nodules (granulomatous-like) throughout the liver, spleen and kidneys (Figs. 1- 4).

The histopathological findings, in the experimentally infected Nile tilapia with the Ph3 isolate of *Photobacterium*, during the acute stage, revealed neuronal degeneration and focal malacia of the brain. Satelletosis and neuronophagia were evident together with focal gliosis (Fig. 5). The heart revealed myocardial edema, hyaline degeneration and Zenker's necrosis with focal infiltration of mononuclear cells. The liver displayed vacuolar degeneration of most hepatic cells with pyknosis of their nuclei. Congestion of the hepatic vessels was evident and some erythrocytes were hemolysed. The pancreas exhibited peri-glandular edema and the pancreatic acinar cells showed more eosinophilic granular cytoplasm with some peri-glandular mononuclear cell infiltrations. The spleen showed degeneration and necrosis of melanomacrophage center with focal lymphoid depletion. The kidneys revealed mild tubular degeneration. In some chronic cases, the liver showed focal necrosis and granulomatous reaction (Fig. 6). The intestine exhibited focal epithelial desquamation with mononuclear cell infiltrations in the lamina propria and edema in the submucosa. The renal epithelium showed vacuolation and focal depletion in the hematopoietic tissues was seen (Fig. 7).

The histopathological changes, in the experimentally infected Common carp with the Ph3 isolate of the *Photobacterium*, during the acute stage, showed marked neuronal degeneration with focal gliosis. Congestion in the meningeal and cerebral vessels was seen and focal malacia was evident in the brain (Fig. 8). The liver revealed vacuolar degeneration of the hepatocytes that contained pyknotic nuclei. Some hepatic cells showed karryolytic nuclei. Most of the pancreatic acini revealed cellular degeneration with deeply stained cytoplasm. The heart revealed myocardial edema, hemorrhage and hyaline degeneration (Fig. 9). The kidneys showed tubular nephrosis mainly vacuolar degeneration, other tubules were necrotic. In some chronic cases, hyaline casts were seen in the renal tubules. Degeneration and necrosis of some hematopoitic cells were seen. The spleen showed focal lymphoid depletion especially in the subcapsular area. The splenic parenchyma displayed focal to diffuse hemosiderosis. Some of the blood vessels contained hemolysed erythrocytes (Fig. 10).The intestine showed numerous mononuclear cell infiltration in the lamina propria and submucosa.

DISCUSSION

Photobacteria strains have been isolated most commonly from the marine environments, surfaces of fishes and marine mud (Austin and Austin 1987 and Plumb 1994). Recently, some authors reported that the *Photobacterium damselae subsp. Piscicida* is the causative agent of the fish pasteurellosis (Thyssen *et al.*, 1998, Osorio *et al.*, 2000, Thyssen *et al.*, 2000, Ahmed, 2002 and Kijima *et al.*, 2007). Moreover, *Photobacterium damselae subsp. damselae* are incriminated as the cause of disease in both of wide range of fish species, in addition, it may be a primary pathogen for mammals, including human where induce wound infection and fatal disease (Labella *et al.*, 2006 and Vaseeharan *et al.*, 2007). *Photobacterium damselae subsp. damselae*, is a systemic bacterial infection affecting more than 48 fish species in widely distributed regions (Santiago *et al.*, 2004). In the current study, it was isolated from *Mugil cephalus*, *Mugil capito* and *Nile tilapia* that reared in wild and cultured environment at Alexandria, Egypt.

Water quality during this study revealed an increase in the unionized ammonia and decrease in the dissolved oxygen as well as decrease in temperature which may play role in the epizootiology of the *photobacteriosis* infections in cultured and wild fish, and these results was contributed with the results obtained by Stephens *et al.*, (2006). Any how, the water quality could be the major stressor in the environment for this type of infection (Plumb, 1994).

The changeable morphology, nutritive requirements, physiology, and biochemistry of the microorganisms made their taxonomic classification and the study of their pathogenic role in fishes extremely difficult. The two subspecies (*piscicida* & *damselae)* of the *Photobacterium damselae* grow on BHIA but only the subspecies *damselae* grow also on TCBS-1. This is the first time to isolate and identify the *Photobacterium damselae subsp. damselae* from the diseased freshwater fish in the Egyptian environment. In the current study, the Ph1, 2 and 3 isolates were found to resemble *Photobacterium damselae subsp. damselae* as described in both the Ninth Edition of Bergey's Manual (1984) and Whitman (2004) where these subspecies have biochemical and physiological traits such as motility, gas production from glucose, nitrate reduction, urease, lipase, amylase and hemolysin production, and a wide range of temperature and salinity for growth. This bacteria was recovered from the wound infections and fatal disease in a variety of marine animals (fish and shellfish) and also from humans as a holophilic bacterium (Osorio *et al.*, 2005 and Vaseeharan *et al.*, 2007). The mechanism by which these isolates could induce the observed clinical

signs and mortalities has been partly attributed to the production of a powerful cytolysin and exotoxins (Vaseeharan *et al.*, 2007).

A total of 50 *Photobacterium damselae subsp. damselae* isolates were obtained from *M. cephalus* (Ph1) with ratio of 24%, *M. capito* (Ph2) with ratio of 20% and *Nile tilapia* (Ph3) with ratio of 56%. These findings considered as a first record to this bacteria from freshwater fish, where it was reported only in seawater (Vaseeharan *et al.*, 2007), marine sea bream and marine seabass (Thyssen *et al.*, 1998) and natural inhabitants of marine, estuarine and aquaculture system of black tiger shrimp *Penaeus monodon* (Vasseharon *et al.*, 2007).

The clinical picture of the reported disease among fishes, in this study, included mainly skin darkening, discoloration, mild hemorrhages at the caudal fin and operculum as well as dorsal musculature, while the postmortem lesions were white nodules on the kidney, liver and spleen. Many of the published reports on Piscine *photobacteriosis* were described the same clinical signs in cultured and wild caught spotted rose snapper (Lutjanus guttatus Steindachner) (Stephens et al., 2006) and hybrid striped bass (Ahmed *et al.*, 2007). Although, the clinical signs and postmortem lesions induced by *photobacteriosis* during this study was mild and not similar to the recorded severe signs in the marine fish or shrimp farms, this results may be attributed to the host susceptibility of the marine and shrimp culture to the *photobaterium damselae subsp. damselae.*

The different aspects of *photobacteriosis* pathogenesis are still obscure (Thyssen *et al.*, 2000), the *photobacterium damselae subsp. damselae* tend to induce kidney, spleen and liver lesions in all of the infected marine fish and marine shrimp. In our study, we could isolate this organism from the liver, kidney and spleen of freshwater fishes which meantime showed marked gross and histopathological changes.

The evaluation of the half lethal concentration dose revealed that, the LD_{50} values was $10^{-4.5}$ CFU per fish for *O. niloticus* and $10^{-2.5}$ CFU and *Cyprinus carpio* to the Ph3 isolate. The LD_{50} values indicated a prime pathogenicity to cyprinids followed by *O. niloticus*. This constitutes a hazard particularly in polycultured fish farms, where common carp is stocked with Nile tilapia to achieve a maximum utilization of the pond primary and secondary productivity (Haas, 1982). Plumb (1994) found that, the *P. Piscicida* can be highly pathogenic, but this varies from species to species. An injectable LD_{50} of $10^{-1.9}$ CFU per ml was established in Tormosa snakehead by Tung *et al.*, (1985), but in other experiment greater numbers of organisms was required to kill fish. Nakai *et al.*, (1992) determined the LD_{50} of this infection in striped Jack (10^3 CFU

per fish) and in red seabream (of 10^7 CFU per fish). The isolates showed high virulence to their non original hosts, as measured by LD_{50} values. The mechanism by which these isolates could induce the observed mortalities is not clear hence, further epizootiological studies on this bacterium should be carried out.

The observed resistance of the recovered photobacteria isolates to most commonly used antimicrobial drugs complicate the selection criteria for chemotherapy during the disease and that conclude the importance of antimicrobial sensitivity test application before the therapy recommendation. Other studies were done on the sensitivity of *photobacterium damselae subspecies damselae* isolates to antimicrobials and showed their sensitivity to tetracycline, ciprofloxacin and sulphamethoxazole plus trimethprin (Stephens *et al.*, 2006).

Histopathological examination revealed degenerative changes during septicemia (acute stage), while large focal area of necrosis in the hepatocytes and splenic lymphoid tissue were observed during the chronic stage of the disease. This picture was described by Ahmed (2002) in hybrid striped bass infected with *Photobacterium damselae subspecies piscida* and Labella *et al.*, (2006) in cultured red banded seabream, Pagrus auriga infected with *Photobacterium damselae subspecies damselae*. Vaseeharan *et al.*, (2007) attributed the pathogenic effect of *Photobacterium damselae subspecies damselae* to the production of a powerful cytolysin and exotoxins which attack the internal organs. However, the current reactions in the paranchymatous organs may be attributed to the influence of bacteria and/or its toxins.

We conclude that, three isolates of *Photobacterium damsellae subspecies damselae* could be recovered from wild and cultured freshwater fish *(Mugil cephalus, Mugil capito* and *Nile tilapia)* and show variable virulence at Alexandria as a new recorded pathogen in our environment.

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مرض الفوتوبكتيريا في أسماك المياه العذبة النهرية والمستزرعة في مصر

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تم تجميع مائتان وسبعون من الأسماك المستزرعة والنهرية (البوري ، الطوبار ، البلطي النيلي) من الإسكندرية وفحصت لعزل الفوتوبكتيريا وقد أختبرت البكتيريا المعزولة لمدى ضراوتها وإحداثها للمرض ومدى حساسيتها لمضادات الميكروبات ، كما تم تحديد خط سير المرض عن طريق الفحص الهستوباثولوجي، و تم تحليل المياه المتواجدة في تلك البيئات. وقد عزلت ثلاثة عترات (1 ، 2 ، 3) من الفوتوبكتيريا دميسله دميسله من البوري والطوبار والبلطي النيلي على التوالي، وقد سبب العترتان رقم 1 ، 2 نسبة نفوق بالأسماك (البلطي النيلي والمبروك العادي) وصلت إلى 20% ، أما العترة الثالثة (رقم 3) فقد أحدثت 40% ، 30% نفوق في البلطي النيلي والمبروك العادي على التوالي ، وقد لوحظ أن الإصابة بهذه البكتيريا كانت مصاحبة بزيادة في الأمونيا الغير متأنية (0.08 ملجم/لتر) ونقص في الأكسجين المذاب (1.8 ملجم/لتر) وزيادة في الأس الايدروجيني (9.8) ، وقد نتج عن العدوى التجريبية ظهور دكانة في الجلد وانزفة على زعنفة الذيل وغطاء الخياشيم ، وبالفحص التشريحي وجدت درنات بيضاء اللون في حجم رأس الدبوس على أسطح الكبد والطحال والكلى ، وبالفحص الهستوباثولوجي فقد اتسمت الإصابة الحادة بالفوتوبكتيريا بوجود تغييرات هدامة في الأعضاء البرانشيمية للأسماك المصابة مع وجود آفات مرضية واضحة بالمخ ووصول التأثير لجهاز تخليق الدم ، أما الإصابة المزمنة للمرض فقد اتصفت بتكوينات درنية مزمنة ونخر بؤرى في الأعضاء الداخلية. وقد كانت الثلاثة عترات المعزولة من الفوتوبكتيريا حساسة للارثرومايسين والاستربتومايسين والامبسيلين ولكنهم كانوا مقاومين كلية للكلورامفينكول والاوكسي نتراسيكلين وحمض الأوكزاليك والكاناميسين والسبروفلوكساسين وعلى النحو الآخر فقد كانت العترة رقم (1) حساسة للفيروازوليدين ولكن العترة رقم (3) كانت حساسة للنيومايسين. ومما تقدم فقد تم عزل ثلاثة عترات من الفوتوبكتيريا دميسيلة دميسيلة مختلفة في الضراوة والحساسية لمضادات الميكروبات من أسماك المياه العذبة المستزرعة والنهرية من منطقة الإسكندرية . واعتبر هذا تسجيلاً جديداً لهذا الميكروب في تلك البيئة.