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Photobacterium damselae infection in yellow tail surgeon (*zebrasoma xanthurum*) of Red Sea at Hurghada, Egypt

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Abstract: *Photobacterium damselae* causes photobacteriosis of marine ornamental yellow tail surgeon (*zebrasoma xanthurum*) the disease appeared and spread rapidly in yellow tail surgeon in the indoor aquarium of National Institute of Oceanography and Fisheries (NIOF) at Hurghada (Egypt). The pathogen was isolated from skin lesions in the body, and internal organs namely liver,spleen and kidney of clinically diseased and moribund fish using tryptic soy agar and thio-sulphate citrate bile salt sucrose agar plates. Lethargic, off food, hemorrhagic spots on skin, skin depigmentation, and fin rot were the main clinical signs appeared on the naturally infected fish. All isolates of the bacterium constituted a homogeneous phenotypic group and were identified by morphological characterization, biochemical tests and API20E as *Photobacterium damselae*. The isolated strain was sensitive to Sulfamethoxazole Gentamycin, and Streptomycin.

Keywords: *Photobacterium damselae*, Photobacteriosis, *zebrasoma xanthurum*, Streptomycin, API20E

1. Introduction

Pasteurellosis or photobacteriosis is a fish disease that causes enormous losses in fish aquaculture production worldwide (Kusuda & Salati 1993, Romalde & Magariiios 1997). The causative agent of fish pasteurellosis is the Gram-negative halophilic bacterium *Photobacterium damselae* subsp, *piscicida,* it was subsequently transferred to the genus *Photobacterium* according to the phenotypic data (Smith *et al*., 1991), and further support was obtained from the phylogenetic analysis carried by Ruimy *et al*. (1994). According to the basis of phylogenetic analysis of 16S rDNA sequences and DNA relatedness the fish pathogen *Pasteurella piscicida,* which causes pasteurellosis in several fish species, was found to be a member of *Photobacterium damselae* (Gauthier *et al*., 1995). *Photobacterium damselae* was initially isolated from white perch and striped bass in the Chesapeake Bay, USA in 1963 (Snieszko *et al.*,1964). In 1969,the pathogen caused great economic losses in cultured yellowtail in Japan (*Seriola quinqueradiata*) (Kubota *et al.*, 1970; Kusuda and Yamaoka, 1972), ayu (*Plecoglossus altivelis*) (Kusuda and Miura, 1972), black sea bream (*Mylio macrocephalus*) (Muroga *et al.*, 1977; Ohnishi *et al.*, 1982), red sea bream (Yasunaga *et al.*, 1983), oval file fish (*Navodan modestus)* (Yasunaga *et al.*, 1984), and red grouper (*Epinephelus okaara*) (Ueki *et al.*, 1990). Moreover, this disease has been reported in the snake-head fish (*Channa maculata*) in Taiwan (Tung *et al.*,1985). In Europe and the Mediterranean, photobacterium was first isolated from the juvenile gilthead sea bream (*Sparus aurata*) in the northwest of Spain in 1991 (Toranzo *et al.*, 1991).in Egypt *photobacterium damsel* was isolated from *Mugil cephalus*, *Mugil capito* and Nile tilapia (Reyad and Salah,2008). The clinical findings of the diseased fishes were haemorrhagic at fins bases, peripheral site of genital pore, and bilateral surface of the abdomen. Additionally, we discovered whitish-mucus gills, edema of the intestines, and multi-focal whitetubercles in infected fishes during gross examination.(Liu *et al.*,2011). Anorexia with darkening of the skin as well as focused necrosis of the gills are the only external clinical signs often observed in some cases(Barber and Swygert,2000) . Affected fish had gas distended swim bladders, anaemia, and the intestines were diffusely distended with a clear, pale yellowish fluid. Livers were mottled tan and green in a zonal pattern(Stephens *et al.*,2006).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 with 40 and 30% mortality among *Oreochromis niloticus* and *Cyprinus carpio* respectively (Reyad and Salah, 2008). In this study we found that photobacteriosis was able

to cause serious infection, even death, in the yellow tail surgeon fish, (*zebrasoma xanthurum)* in indoor aquarium of National Institute of Oceanography and Fisheries at Hurghada Egypt. Morphological and biochemical identification, antibiotic sensitivity test and virulence of the isolates are described.

2. Materials and Methods

2.1. Fish

Thirty clinically diseased and moribund yellow tail surgeon fish (*zebrasoma xanthurum*) were collected from the indoor aquaria of The National Institute of Oceanography and Fisheries (NIOF) at Hurghada and subjected to clinical examination and bacteriological isolation according to Liu *et al*.,2011.

2.2. Water Samples

Water samples were collected from the investigated indoor aquarium and the red sea (control sample) in dark brown clean and dry bottles. Water temperature and pH were determined by thermometer and digital combo pH meter (HI 98127 (pHep 4) -Hanna instruments Inc., USA), total ammonia was determined and dissolved oxygen (DO) concentration were measured using a digital dissolved oxygen meter (HI 9142 - Hanna instruments Inc., USA).

2.3. Bacterial Isolation and Characterization

Samples for bacterial isolation were taken from skin ulcers, liver, spleen and kidney of moribund and clinically diseased yellow tail surgeon fish, (*zebrasoma xanthurum*) and cultured on plates of tryptone soya agar (Oxoid) supplemented with 1.5% (w/v) sodium chloride (TNA) . The inoculated plates were incubated at 28°C for up to 72 hrs .The suspected *P.damselae* colonies were isolated, purified using thiosulphate citrate bile salt sucrose agar (TCBS, Difco) and characterized and identified according to standard morphological, physiological, biochemical method and Commercial miniaturized API 20E galleries (BioMerieux) (Gauthier *et al.,* 1995; Abbasi *et al.,* 2010; Liu *et al.,*2011)

2.4. Antibiotic Sensitivity Assay

The bacteria were grown on TSA at 28°C for 24 h. Then the bacteria were suspended in sterile phosphate buffered saline [PBS] and diluted as the MacFarland No. 0.5 standard solution tube (0.5 mL BaSO4 + 99.5 mL 0.36 N HCl), about 1 × 107 CFU/mL. The bacterial suspension (0.1 ml) was spread onto Mueller-Hinton agar (Difco) and antibiotic discs then added as described by Koneman *et al.* (1988). and the following antibiotics streptomycin, chlormphenicol, oxytetracycline, oxolinic acid, ciprofloxacin, getamicin, Ampicillin, sulfamethoxazole and kanamycin, were used (Oxoid). The tested plates were incubated at 28°C for 18 h. The results were then interpreted and recorded according to Koneman *et al.,*(1988).

2.5. Fish Pathogenicity Experiments

Twenty (20) *yellow tail surgeon* (*zebrasoma xanthurum*) fish were acclimated for one week in the indoor aquarium and subdivided into two equal groups each of 10 fish (weighing 110± 10 g. each) and held in a tank (100 liter) for testing. Each fish in the first group received intraperitoneal (i.p.) injections with0.1 mL/fish of bacterial suspension to achieve doses of 10^6 cells $fish^{-1}$ (Toranzo *et al.* 1983). The second group inoculated with sterile PBS by i.p. served as the parallel control. The clinical signs and mortalities were monitored and recorded daily for 14 days after the shots. Re-isolation and identification of the bacteria from the inoculated fish was also performed.

3. Results

3.1. Clinical Signs

The clinical signs of the diseased *zebrasoma xanthurum* fish were lethargic, off food with depigmentation of the skin of the infected fish. Skin hemorrhagic spots and fin rot were also recorded (Figure – 1 and2). The main post mortem lesions were pale yellowish fluid in the abdominal cavity, the livers were mottled in a zonal pattern with presence of small nodules also congestion and adhesions of the internal organ was recorded (Figure –3 and4). The recorded mortality among the diseased fish was 60%..

Figure (1). *Yellow tail surgeon (zebrasoma xanthurum) fish showed Skin hemorrhagic spots and fin rot.*

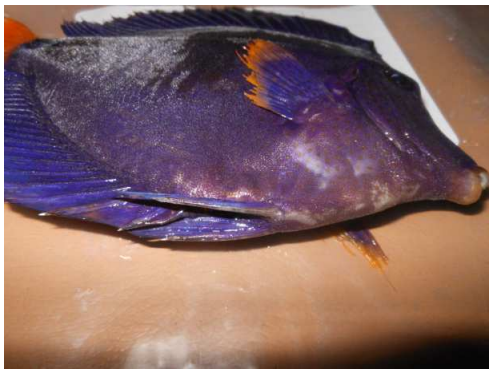

Figure (2). *Yellow tail surgeon (zebrasoma xanthurum) fish showed Skin depigmentation and fins rot.*

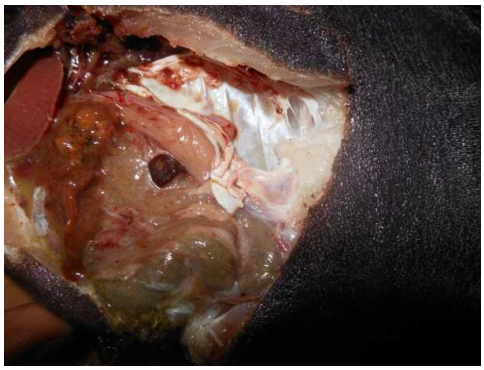

Figure (3). *Yellow tail surgeon fish show presence of liver nodules also congestion and adhesions of internal organs*

Figure (4). *Yellow tail surgeon fish showed liver were mottled in a zonal pattern.*

3.2. Water Quality

The results of this study revealed elevation of ammonia and pH values while the dissolved oxygen was decreased in the water samples of indoor aquarium, table (1).

Table 1. *Water quality criteria.*

Item	Unit	Tested sample	Control sample
Water temperature	°C	26	25
pH values	-	8	7.5
Dissolved oxygen	mg L¯1	3.1	5.5
total ammonia	mg L¯1	0.0054	0.00038

3.3. Bacterial Characterization

Nine bacterial isolates were isolated from skin lesions, liver, kidney, and spleen. These colonies of presumptive *Photobacterium damselae* were rounded viscous, regular shiny-grey-yellow in color. The biochemical and physiological characteristics of all the isolates were similar and allowed the presumed identification of the bacteria as *Photobacterium damselae*. In fact the staining characteristics of our pleomorphic rod-shape isolate were Gram-negative with bipolar staining, non-motile, oxidase and catalase positive. They were negative in Vogues-Proskauer and lysine decarboxylase tests but positive in arginine dehydrolase, and urease tests. All bacterial isolates produced acid from carbohydrates fermentation test (D-glucose and D-mannitol) but no gas produced. However, no acid was produced from other carbohydrates such as D-lactose, D- Arabinose, D-raffinose,and L-rhamnose (Table 2). All bacterial isolates grew well at 25-35 °C on TCBS agar with green colonies and all bacterial isolates grew well in 1.5-6 % (w/v) sodium chloride but not in 0 % and 8 %, respectively.

Table 2. *Results of the biochemical characterization of the Photobacterium damselae isolates*

Tests	Result	Tests	Result
Colony shape	Round	Colony colour	Viscous yellow
Gram stain	-ve rods	Motility	+ ve
Cytochrome oxidase	+ve	Catalase	+ ve
Growth in 0% NaCl	-	Growth in 1.5% NaCl	+
6% NaCl	+	8% NaCl	-
nitrate	+	0/129 disk	+
API20E			
ONPG	-	GEL	-
ADH	+	Glucose	+
LDC	+	Manitol	+
ODC	-	Inositol	-
CIT	+	Sorbitol	-
H2S	-	Rhaminose	-
URE	+	Sucrose	v
TDA	-	Malonate	-
IND	-	Adonitol	-
VP	+	Arabinose	-
Raffinose	-	Salicin	-
Xylose	+	Lactose	-

ODC = ornithine decarboxylase, LDC = lysine decarboxylase, ADH = arginine dihydrolase, IND = indole, CIT = citrate, URE = urea hydrolysis, VP = Voges-Proskauer, TDA =tryptophane deaminase , GEL = gelatin hydrolysis, ONPG= Ortho -nitrophenyl b-d-galactopyranoside, H2S = hydrogen sulfide production.

3.4. Antibiotic Sensitivity Assay

The antimicrobial sensitivity test revealed that the isolated strain was sensitive to Sulfamethoxazole Gentamycin, and Streptomycin. Controversially, it was resistant to Ampicillin, Ciprofloxacin, Chloramphenicol, Kanamycin, Oxytetracycline And Oxolinic acid (table 3).

Table 3. *Sensitivity of Photobacterium damselae isolates to various antibiotics.*

Antibiotics	Result
Ciprofloxacin	R
Chloramphenicol	R
Streptomycin	S
Kanamycin	R
Oxytetracycline	R
oxolinic acid	R
Ampicillin	R
getamicin,	S
sulfamethoxazole	S

S = sensitive R = Resistant

3.5. Fish Pathogenicity Experiments

The virulence assays with *Photobacterium damselae* isolates demonstrated that the experimentally infected *yellow tail surgeon* fish showed lesions similar to those of naturally infected fish, lethargic, off food, with depigmentation of the

skin and, hemorrhagic spots on the skin. The observed PM lesions revealed the presence of congestion of the visceral organs and ascitis. By the end of observation time (14 days) the cumulative mortality of the experimentally infected fish reached 70 %. *Photobacterium damselae* was re-isolated in pure culture from the experimentally infected fish.

4. Discussion

Photobacteriosis is caused by the halophilic bacterium *Photobacterium damselae subsp. piscicida* (formerly *Pasteurella piscicida*). The detection of *Photobacterium damselae* infection in yellow tail surgeon in the present condition favoring the establishment of photobacteriosis by this highly pathogenic halophilic organism. This pathogen has proven to be detrimental to wild and aquarium fishes, and is responsible for severe losses of cultured yellowtail juveniles (Kusuda and Yamaoka, 1972), gilthead sea bream outbreak in northwestern Spain in 1990 (Toranzo *et al., 1991)* , Ayu, Red sea bream, Black sea bream and Red grouper in Japan (Acosta *et al.,* 2006; do Vale *et al.,* 2005). The clinical signs of *Photobacterium damselae* infection in yellow tail surgeon fish were lethargic, off food with depigmentation of the skin of the infected fish, skin hemorrhagic spots and fin rot. Similar observations were noticed by Stephens *et al., (*2006), Reyad and Salah, (2008) and Liu *et al.,(*2011). The clinical signs of photobacteriosis were noticed on the wild investigated yellow tail surgeon within few days after fishing and rearing in the indoor aquarium. The onset of the disease may be resulted from the suppression of the fish immune system due to increased ammonia and pH level and decreased dissolved oxygen in addition to the over crowdedness in the indoor aquarium. This explenation was supported by Bullock *et al.,*(1986)and Suomalainen *et al.,* (2005). The Postmortem lesions revealed presence of pale yellowish fluid in the abdominal cavity, livers were mottled white and green in a zonal pattern also congestion and adhesions of internal viscera. Similar postmortem lesions were recorded by agreement with the findings of Belen *et al.,* (1992), Stephens *et al.(,*2006), Reyad and Salah,2008 and Labella *et al.,(* 2011). The Nine bacterial isolates from naturally infected fish during mortality episodes were identified as *Photobacterium damselae* by the colony characters, cell morphology, gram stain and biochemical reactions including the API20E tests. This finding was in agreement with the result of Toranzo *et al.,* (1991), Nicky (2004), Reyad and Salah (,2008)and Liu *et al.,(*2011). The antibiotic sensitivity test of *Photobacterium damselae* showed that the isolated strain was sensitive to Sulfamethoxazole Gentamycin, and Streptomycin. Controversially, it was resistant to Ampicillin, Ciprofloxacin, Chloramphenicol Kanamycin, Oxytetracycline And Oxolinic acid. As such, the study mor or less ingreement with Labella *et al.,(*2011) Reyad and Salah(,2008).The high resistance of the recovered photobacteria isolates to most commonly used antimicrobial drugs proven the importance of antimicrobial sensitivity test application before the therapy (Stephens *et al*., 2006).The experimentally infected yellow tail surgeon fish showed lesions similar to those of naturally infected fishes such lethargic, off food , with depigmentation of the skin and, hemorrhagic spots on the skin. associated with 70% mortality and similar clinical signs were reported by Toranzo *et al.,* (1983) Reyad and Salah, (2008). The pathogenicity of *P. damselae* may be attributed to the extracellular product mainly damselysin, a thermolabile extracellular cytotoxin of 69 kDa, which is a phospholipase D active against the sphingomyelin of the sheep erythrocyte membrane and has haemolytic activity against several erythrocyte types, including fish (Kothary & Kreger, 1985; Cutter & Kreger, 1990 and Kreger *et al.,* 1987) . In conclusion *Photobacterium damselae* is the causes *zebrasoma xanthurum* mortality and its importance as a pathogen in salt water aquaculture is being increasingly recognized. Therefore it may become necessary to conduct further studies toward vaccination and molecular characterization.

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