

# SCREENING OF *Photobacterium* sp., A POTENTIAL PATHOGEN IN THE FISH LANDED AT PULICAT LAKE, TAMIL NADU, INDIA

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## ABSTRACT

Fish are the source of high-quality protein and are preferable due to its exceptional richness in calcium, phosphorus and vitamins. *Photobacterium damsela* is a potential pathogen of marine fish and an opportunistic pathogen in humans which causes pasteurellosis. A study for the screening of *Photobacterium* sp. in the marine fish landings of Pulicat Lake, Tamil Nadu, India was undertaken between August 2018 and February 2019. About 113 samples of fishes belonging to various species viz., *Ablenneshians*, *Rastrelliger kannagarta*, *Hemiramphus*, *Thunnus* sp., *Nemipterus bipunctatus*, *Leognathus* sp., *Scombroid* sp., *Saurida thumbil*, *Triacanthus biaculatus* and *Pomadysys* sp. were used for the study. *Photobacterium* sp. has been isolated in 26 fish samples which include *Rastrelliger kannagarta* (13/16), *Leognathus* sp. (4/11) and *Saurida thumbil* (9/11). Pathogenic *Photobacterium* sp. could pose threat to the health of consumers through the ingestion of contaminated, improperly cooked seafood or may gain entry to the human body during handling of contaminated fish through the cuts or abrasions or by swimming and other recreational activities in the contaminated water bodies.

**Keywords:** Capsular polysaccharide (CPS) gene, Opportunistic pathogen, *Photobacterium damsela*

Received : 08.04.2022

Revised : 30.05.2022

Accepted : 08.07.2022

## INTRODUCTION

*Photobacterium* sp. is a luminous and non-luminous bacterial pathogen infecting marine and brackish water fish, animals and human beings (Hawke *et al.*, 2003). The genus *Photobacterium* belongs to the family,

Vibrionaceae (Shieh *et al.*, 2003) which are gram-negative, motile, oxidase positive, able to grow in aerobic or anaerobic environments at 37°C, capable of reducing nitrogen and exhibit haemolytic activity (Kaysner, 2000; Molin, 2000). *P. damsela*, formerly known as *Vibrio damsela* was originally isolated from the skin ulcer of Damsel fish (Love *et al.*, 1981). It is considered as a zoonotic agent causing necrotizing fasciitis (Hawke, 2012), infecting a variety of marine animals including fish, crustaceans, molluscs, and cetaceans (Rivas *et al.*, 2013). Recent findings demonstrate

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that the phospholipase-DDly (damselysin) and the pore-forming toxins HlyApl and HlyAch play a main role in the virulence of *P. damsela* (Rivas *et al.*, 2013) *Photobacterium damsela* subsp. *piscicida*, (formerly known as *Pasteurella piscicida*) is considered an obligate pathogen and it is short-lived outside the host even in saltwater (Hawke, 2012). *P. damsela* subsp. *damsela* and *P. damsela* subsp. *piscida* have a high level of similarity at the 16s rRNA gene sequence but they can be distinguished by phenotypical traits (Botella *et al.*, 2002). Polysaccharide capsular layer plays a major role in the virulence of the pathogen and also has extracellular products and iron availability (Magarinos *et al.*, 1994). The objective of the study is to screen for the pathogenic *Photobacterium sp.* in the marine fishes landed at Pulicat Lake, Tamil Nadu, India.

## MATERIALS AND METHODS

### Sampling and bacteriology

A total number of 113 fish samples represented by needlefish (*Ablenneshians*) -12 Nos, mackerel (*Rastrelliger kannagarta*)-16 Nos, garfish (*Hemiramphussp.*) - 9 Nos, tuna fish (*Thunnus sp.*)-10 Nos, seabreams (*Nemipterus bipunctatus*) – 10 Nos, pony fish (*Leognathus sp.*) – 11 Nos, *Scombroid sp.*, -14 Nos, lizardfish (*Saurida thumbil*)-11 Nos, tripodfish (*Triacanthus biaculatus*)- 9 Nos and grunts (*Pomadasys sp.*)-11 Nos were collected during the period from August 2018 to February 2019 from the fish landings of Pulicat brackish water lake, Tamil Nadu (13.455474, 80.279172). Screening and isolation of *Photobacterium sp.* was carried out from the internal organs viz. liver,

kidney, gill, brain, skin and intestine of fish which were first rinsed in sterile saline and dissected aseptically. Inocula from the organs were collected aseptically and streaked on to thiosulfate citrate bile salt sucrose agar (TCBS), tryptic soy agar (TSA) with 1.5% NaCl and incubated at 22°C for 24–48 hrs. Based on the dominance and definite colony morphology (59 isolates), representative colonies from the media were picked, purified on TSA and maintained in glycerol stock at –70°C for further characterization.

### Phenotypic characterization of bacterial strains

A series of biochemical tests viz., oxidase, catalase, H<sub>2</sub>S production, indole, oxidative fermentation, nitrate reduction, Voges–Proskauer test, methyl red, citrate, sensitivity test to O/129(10g), ornithine, urease, lysine, glucose, adonitol, lactose, arabinose and sorbitol were performed to identify the bacterial strain up to the genus level. The phenotypic characteristics documented in earlier reports (Holt *et al.*, 1994 and Brenner *et al.*, 2005) were compared for presumptive identification of the isolate. The presumptive isolates were further subjected to PCR amplification.

### Molecular characterization

The genomic DNA was extracted from the presumptive isolates using QIAamp genomic DNA kits (Qiagen, Germany) as per manufacturer's protocol. PCR amplification of a fragment of *Photobacterium*-specific capsular polysaccharide (CPS) gene (Rajan *et al.*, 2003) and 16S rRNA primer Car1 and Car2 (Osorio *et al.*, 1999) were carried out

in a T100™ thermal cycler (Bio-Rad, USA) following the PCR protocols. The PCR reaction mixture contained 1µl of genomic DNA, 5 µM of each primer and 2X PCR Taq-Mixture (Ampliqon, Denmark). PCR products were analyzed on a 1.5% agarose gel containing 0.5 µg/mL ethidium bromide in 1x Tris-borate-EDTA (TBE) buffer and visualised under UV illumination using a gel documentation system (Biorad, USA). The amplified PCR products were purified and sequenced using both forward and reverse primers (Eurofins, Bangalore, India). The sequence similarity was analysed following NCBI BLAST (<http://blast.ncbi.nlm.nih.gov>).

## RESULTS AND DISCUSSION

From the 113 fish samples (*Ablenneshians*, *Rastrelliger kannagarta*, *Hemiramphus*, *Thunnus* sp., *Nemipterus punctatus*, *Leognathus* sp., *Scombroid* sp., *Saurida thumbil*, *Triacanthus biaculatus* and *Pomadasy* sp.) collected from Pulicat Lake, about 75 bacterial isolates were obtained. Further, biochemical characterization was performed following various tests *viz.*, oxidase, catalase, nitrate reduction, H<sub>2</sub>S production, Voges–Proskauer, methyl red, urease, oxidative fermentation, Indole, citrate utilization, ornithine decarboxylase, lysine decarboxylase, fermentation of glucose, adonitol, lactose, arabinose, and sorbitol and O/129 sensitivity test. About 46 isolates presumptively identified as *Photobacterium* sp. were positive for oxidase, catalase, nitrate reduction, H<sub>2</sub>S production, Voges–Proskauer, methyl red, urease, oxidative fermentation and negative in the test for Indole, citrate utilization, ornithine decarboxylase, lysine

decarboxylase and fermentation of glucose, adonitol, lactose, arabinose and sorbitol and also sensitive to O/129. These presumptive isolates were obtained from 26 fish samples belonging to Mackerel, *Rastrelliger kannagarta* (13/16), ponyfish, *Leognathus* sp. (4/11) and lizard fish, *Saurida thumbil* (9/11). The prevalence of *Photobacterium* sp. in the total fish samples collected was 23% (26/113\*100). Confirmation of the bacterial isolates were done by PCR amplification of capsular polysaccharide (CPS) and 16S rRNA gene which resulted in amplified products of 410 bp and 267 bp respectively. On the basis of gene sequence similarity, the strain was identified as *Photobacterium damsela* and a homogeneity of 99.03 to 98.48% was observed with other such species of the genus and 98.67 to 97.29% similarity with other bacteria in the family (Accession number: MK284529, MK250484 and MZ093189).

*P. damsela* is a common pathogen of wild and captive fish with two sub-species *viz.* *P. damsela* subsp. *damsela* and *P. damsela* subsp. *piscicida* (Romalde, 2002). *P. damsela* subsp. *damsela* infection has been reported in a wide variety of aquatic animals. The disease has been reported in cultured fishes *viz.*, rainbow trout (*Oncorhynchus mykiss*), ovate pompano (*Trachinotus ovatus*), sea bass (*Dicentrarchus labrax*), yellowtail (*Seriola quinqueradiata*), red banded seabream (*Pagrus auriga*) (Pedersen *et al.*, 2009; Zhao *et al.*, 2009; Labella *et al.*, 2010), Striped sea bass (Nitzan *et al.*, 2001), cage-farmed cobia (*Rachycentron canadum*) (Sharma *et al.*, 2016) and *Penaeus monodon* (Vaseeharan *et al.*, 2007). In wild fishes, the disease has been reported in octopus, *Octopus joubin* (Hanlon

*et al.*, 1984), turtles, *Dermochelys coriacea* (Obendorf *et al.*, 1987), dolphins, *Tursiops truncatus* (Fujioka *et al.*, 1988), Grouper, eight b and butterfly fish, wild parrotfish, *Lutjanus sp.* (Petchimuthu *et al.*, 2021) and cetaceans (Wang and Chen 2006). Disease caused by *P.damselae* subsp. *damselae* has also been documented in wound infections in humans (Love *et al.*, 1981; Clarridge *et al.*, 1985). Disease outbreaks due to *P.damselae* subsp. *damselae* have been documented in various aquaculture systems *viz.*, earthen ponds, net pens and sea cages as well as indoor tanks and aquaria. The pathogen is known to be transmitted through the water and highly susceptible fish species may be infected by this route (Hawke, 2012). The clinical signs exhibited by the fishes with *P. damsela* subsp. *damsela* include external skin ulceration that may progress to haemorrhagic septicemia. Systemic infection with the pathogen caused fatty liver with petechiae, abdominal swelling from ascites and splenomegaly (Labella *et al.*, 2011). *P. damsela* strains have a high-affinity siderophore-mediated iron-sequestering system that leads to an uptake of iron from the affected fishes leading to signs of anemia (do Vale *et al.*, 2002). In addition production of extracellular products (ECPs) as virulence factors results in haemolytic and phospholipase activities that occur in haemorrhage (Labella *et al.*, 2010, Hundenborn *et al.*, 2013). In summary, this paper has highlighted the presence of potentially pathogenic species of *Photobacterium damsela* infection in the fishes landed in the Pulicat lake, Tamil Nadu. Hence it is essential to follow hygienic and safe handling procedures in fish landing centre to avoid the transmission of *P.damsela* subsp. *damsela* to the human handlers.

## ACKNOWLEDGEMENT

The authors thank Tamil Nadu Dr.J.Jayalithaa Fisheries University for extending the facilities for this research work. We thank Tamil Nadu State Planning Commission for their funding support to carry out this research as a part of E-fish health surveillance project through Tamil Nadu innovation initiative (TANII) scheme.

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