Indian J. Fish., 64(4): 71-79, 2017
DOI: 10.21077/ijf.2017.64.4.71261-10

Isolation and characterisation of virulent *Serratia marcescens* associated with a disease outbreak in farmed ornamental fish, *Poecilia reticulata* in Kerala, India

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ABSTRACT

Pathogenic strain of *Serratia marcescens* (NPSM-1) with multiple drug resistance was isolated from guppy *Poecilia reticulata* with clinical signs of fin rot and was confirmed by biochemical tests and 16S rRNA gene sequencing. The extra cellular proteins (ECP) of the bacteria exhibited marked cytotoxic activity *in vitro* on *Cyprinus carpio* koi fin (CCKF) cell line. The *in vivo* challenge studies confirmed that the isolate was highly pathogenic to fish when the fishes were injected with 1 x 10^4 CFU/fish and the same bacterium was re-isolated from infected fish, post-challenge. *S. marcescens* produced large zones of haemolysis on 10% sheep blood agar. The bacteria was found to carry virulence genes; extracellular metallo-protease gene (*Pr596*) and AHL synthase gene (*Spn*I). The bacterial isolate was tested to determine sensitivity against 16 antibiotics and was sensitive to only 5 *viz.,* cefixime, chloramphenicol, ciprofloxacin, gentamycin and erythromycin. The study indicates that *S. marcescens* can cause disease in ornamental fish and the bacterium being a known human pathogen, may also cause infections in humans having direct contact with infected fishes. This is the first report describing *S. marcescens* as a pathogen of freshwater ornamental fish in India.

Keywords: Bacterial fish diseases, Guppy, Ornamental fish, *Poecilia reticulata, Serratia marcescens*

Introduction

Ornamental fish culture is one of the important and promising areas of aquaculture worldwide. In India, ornamental fish industry is expanding and is supported by government agencies such as the Marine Products Export Development Authority (MPEDA) (Silas *et al*., 2011). Diseases are inevitable with the rapid expansion of the aquaculture industry and pathogens have become one of the major bottlenecks to production. Variation in microbial community of water in the aquaculture systems is considered to be the major factor causing diseases and mortalities in fishes (Gomes, 1996). Austin *et al*. (1999) reported that higher quantities of organic material, changes in pH values and enhanced microbial populations resulted in infectious diseases in aquaculture.

The members of the family Enterobacteriaceae *viz., Yersinia ruckeri, Edwardsiella tarda* and *E. ictaluri* are recognised as fish pathogens (Sanders and Fryer, 1988). However, other enterobacteria such as *Proteus* sp., *Citrobacter* sp., *Hafnia* sp. and *Serratia* sp. have been associated with fish disease outbreaks (McIntosh and Austin, 1990). The genus *Serratia* comprising *Serratia liquefaciens, S. marcescens* and *S. plymuthica* have been associated with bacterial septicaemia and mortalities in salmonids (Austin and Gibb, 1993), though these species are considered opportunistic pathogens.

S. marcescens is a well known cause of hospital acquired infections, including nosocomial pneumonia, wound infections, urinary tract infections and septicaemia (Yu, 1979) and is a common microorganism present in soil and freshwater (Hejazi and Falkiner, 1997). Until now, there have been only a few publications concerning the fish infections caused by this microorganism. Baya *et al*. (1992) isolated *S. marcescens* from natural populations of white perch. *Morone americanus*, during the course of a bacteriological survey in USA. However, the emergence of multidrug resistant *Serratia* has been alarming not only in the medical field but also in aquaculture and agriculture sectors (Morohoshi *et al*., 2007). Recently *S. marcescens* has been isolated in an endemic disease outbreak from

tilapia fish farms in Malaysia and its whole genome was sequenced (Chan *et al*., 2013). In the course of routine monitoring for diseases in ornamental fishes under the National Programme of Surveillance of Aquatic Animal Diseases (NSPAAD) in India, in June 2015, a farmer reported high mortality and morbidity in guppy, *Poecilia reticulata* with skin and fin rot lesions. The specimens were examined for important bacterial, viral and parasitic infections. We isolated a red pigmented bacterium which resembled *S. marcescens* from the affected fishes sampled from the farm. Because of the possible public health implications due to these bacteria, we aimed to characterise this microorganism; to determine whether *S. marcescens* is really the causative agent of such health disorders in guppy fish; and to select the most suitable antibiotic agent for the treatment of fishes affected by this bacterium.

Materials and methods

Fish sampling

The ornamental fish farmer from Kozhikode, Kerala reported several incidences of mortality in guppies (body weight range : 0.45 to 0.80 g) with fin and tail rot and mortality up to 40% during the rainy season (June-August) in 2015, with a history of not responding to antibacterial, antiparasitic and antiprotozoan treatments. Diseased (n = 25) fish were collected from the farm for detailed investigations. All fish were transported to the laboratory on ice within 6 h. The tissues *viz.,* fin, gills, spleen and kidney from the affected fish were stored in 95% ethanol and Leibovitz's L-15 tissue culture medium for screening of viruses *viz.,* Koi Herpes virus (KHV), Iridovirus and spring viraemia of carp virus (SVCV) as described by Kumar *et al*. (2015) and isolation of viral pathogen, if any, respectively. A tissue homogenate was prepared from the pooled samples of fin, gills, spleen, heart and kidney from the affected fish and inoculated on different fish cell lines *viz.,* pearl spot fin (PSF) (Swaminathan *et al*., 2010), catopra fish fin (CFF) (Swaminathan *et al*., 2013), *Horabagrus brachysoma* fin (HBF) (Swaminathan *et al*., 2014), *Cyprinus carpio koi* fin (CCKF) (Swaminathan *et al*., 2015), angelfish fin (AFF) (Swaminathan *et al*., 2016) and goldfish fin (GFF) (unpublished) maintained in our laboratory to screen for viral infections. The scrapings from skin, fin and gills of the affected fish were collected and examined under microscope for external parasitic infestation.

Bacterial isolation

For bacterial isolation, whole fish was homogenised in sterile phosphate buffered saline (PBS) in the ratio of 1:1 (w/v) and inoculated into trypticase soy agar (TSA; Himedia, India). The cultures were incubated at 28°C for 48-72 h and the number and diversity of colonies were determined. Preliminary tests allowed us to identify the dominant red pigmented colonies isolated from guppy as *Serratia* sp. and biochemical tests were conducted following procedures described by Barrow and Feltham (2004). Pure cultures were kept frozen at -80°C in double strength tryptic soy broth supplemented with 15% glycerol for further examination. For the taxonomic analysis, the reference strains of *S. marcescens* ATCC 1800 and *S. marcescens* isolated from natural populations of white perch, *Morone americana* (Baya *et al*., 1992) were included for comparison.

PCR amplication of 16S rRNA*, metalloprotease and quorum sensing genes of S. marcescens*

S. marcescens isolate was further confirmed by sequence analysis of 16S rRNA gene, extracellular metalloprotease gene (*Pr596*) and AHL synthase gene (*Spn*I) (Tao *et al*., 2007; Tariq, 2010). Total genomic DNA was isolated from pure bacterial cultures using the DNeazy blood and tissue kit (Qiagen) following the manufacturer's instructions. The DNA concentration was quantified with a bio-spectrophotometer (Eppendrof, Germany) and adjusted to a concentration of 100 ng μl^{-1}. Universal primers 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-TACGGCTACCTTGTTACGACTT-3), were used to amplify the 16S rRNA gene (Weisburg *et al*., 1991). The PCR products were sequenced at an automated sequencing facility (Scigenom Pvt. Ltd, India). The raw DNA sequences were edited using BioEdit sequence alignment editor version 7.0.5.2 (Hall, 1999). For molecular identification, homology comparison of 16S rRNA sequences of bacterial strains was performed using Basic Local Alignment Search Tool (BLAST) and Ribosomal Database Project (RDP). The sequences were compared for similarity between the sequences of collected bacterial isolates and the sequences available at GenBank and a phylogenetic tree was constructed by neighbour-joining method. Distance matrices were calculated using Kimura's 2-parameter correction and stability of groupings and bootstrap analysis (1000 replicates) was conducted using MEGA 5.05 software (Tamura *et al*., 2011). *Vibrio cholerae* (GenBank Accession No. LC011458) was used as an out group. Additionally, two sequences of *S. marcescens* previously submitted to GenBank (Accession nos. AY498856 and EF194094) and other *Serratia* sp. *viz., S. liquefaciens, S. plymuthica, S. odorifera, S. glossinae* and *S. ficania* were included in phylogenetic analysis. The partial 16S rRNA, *Pr596* and *Spn*I gene sequences of *S. marcescens* isolate were deposited in the GenBank database.

Heamolytic activity

S. marcescens produces a variety of virulence proteins, including heamolysin, which was reported to be the dominant virulence factor of the bacterium. The strain was tested for haemolysis activity on blood agar containing 10% sheep erythrocytes. Tryptone-yeast extract (TY) base (HiMedia, India) was used to prepare blood agar plates. The bacterial suspensions were streaked on to the plates and plates were evaluated initially after incubation for 24 h at 28°C and after further incubation for 12 h at 4°C. A clear and colourless zone around the colony indicates α-haemolytic activity.

Challenge tests

The bacteria, *S. marcescens* was grown in trypticase soya broth for 24 h at 28°C and then centrifuged at 2000 g for 10 min and cell pellets were suspended in sterile PBS to the final concentration of 1 x 10^{5} cells ml^{-1}. Healthy guppies weighing 0.50 - 0.75 g were used in challenge experiments. Before infection, the fish were anaesthetised with MS-222 (Sigma). For each bacterial strain, 10 guppy fish were injected intraperitoneally (i/p) with 100 µl of the bacterial suspension for testing Koch's postulates and the same number of fishes were injected with sterile PBS, which were treated as the controls. Fishes were maintained in 100 l capacity glass aquaria with 50 l water with continuous aeration, daily 50% exchange of water and *ad libitum* feeding. The challenged fish were observed daily for infection after inoculation with *S. marcescens* bacterial suspension. Infected fish were examined, bacteria reisolated and reconfirmed by biochemical tests, PCR and sequencing as mentioned above.

Antibiotic sensitivity tests

S. marcescens isolates were tested for antibiotic susceptibility using disc diffusion method on Mueller Hinton Agar (Himedia, India) (Bauer *et al*., 1966). A total of sixteen antibiotics (HiMedia, India) were tested: ampicillin (25 µg $disc^{-1}$), gentamycin (120 µg $disc^{-1}$), oxytetracycline (30 µg $disc^{-1}$), cefalexin (30 µg $disc^{-1}$), chloramphenicol (25 µg $disc^{-1}$), ciprofloxacin (30 $µg^{-1}$ disc), cefixime (5 µg $disc^{-1}$), kanamycin (30 µg $disc^{-1}$), nitrofurantoin (100 µg $disc^{-1}$), erythromycin (10 µg $disc^{-1}$), amoxicillin (25 µg $disc^{-1}$), furazolidone (100 µg $disc^{-1}$), bacitracin (10 µg $disc^{-1}$), azithromycin (30 µg $disc^{-1}$), enrofloxacin (10 µg $disc^{-1}$) and cefixime/ clavulanic acid (5/10 µg $disc^{-1}$). Antibiotic sensitivity was assayed from the diameter of zone of inhibition formed around the discs. Manufacturer's instructions were used to determine the sensitivity as sensitive, intermediate and resistant to antibiotics.

Cytotoxicity study

The extracellular product (ECP) of *S. marcescens* was obtained by the cellophane plate technique (Liu, 1957). Briefly, sterile cellophane tape was placed on TSA plates and inoculated by spreading 0.5 ml of broth culture of *S. marcescens* over the surface with sterile swab. After incubation at 28°C for 24 h, bacterial cells were washed off the cellophane with PBS. The cell suspension was centrifuged at 400 g for 30 min at 4°C and the resulting supernatant was filtered using 0.45 µm syringe filter and stored at -80°C until required. The cytotoxicity of ECP was assessed on CCKF cell line (Swaminathan *et al*., 2015) in duplicate in 6-well plates and control wells were inoculated with sterile phosphate buffered saline (PBS). For this purpose, the ECP of the isolate was inoculated and observed at regular intervals (every 6 h) by inverted microscopy for signs of cytotoxicity for up to 7 days post-infection.

Results and discussion

A total number of 20 infected fish from the affected ornamental fish farm were examined in this study. Affected fish were initially examined and most of the individuals showed clinical signs such as emaciation of body, distended anus, discoloration of the body and fin rot. Screening using molecular tools revealed that the fish samples were free from koi herpes virus (KHV), spring viraemia virus (SVCV) and iridovirus. No cytopathic effects (CPE) could be detected in any of the fish cell lines *viz.,* RTF, HBF, CFF, PSF, PFF, CCKF and GFF, inoculated with the tissue filtrate even after 15 days post-inoculation (dpi). Moreover, even occasional parasites such as *Trichodina* sp., *Dactylogyrus* sp. or *Gyrodactylus* sp. were not observed in the affected fish.

Putative characteristic red pigment producing colonies for *Serratia* species were isolated from diseased guppies along with other bacterial colonies. *Serratia marcescens* on TSA (Fig. 1) constituted 85-90% of total bacterial flora in samples from individual fish showing clinical signs of disease and remaining 10-15% colonies were identified as *Bacillus* sp. The characteristics of the isolates were: Gram negative motile rods, fermentative, oxidase and catalase negative, indole negative, Voges-Proskauer and nitrate positive, arginine dehydrolase negative but lysine and ornithine positive with gas production from glucose but not H_2S. Other morphological and biochemical characteristics are described in Table 1. These properties, together with the carbohydrate fermentation pattern, are similar to those exhibited by the reference strain of *S. marcescens* ATCC 8100 and white perch isolate *S. marcescens* (RB 469) (Baya *et al*., 1992). Results of biochemical tests of the red

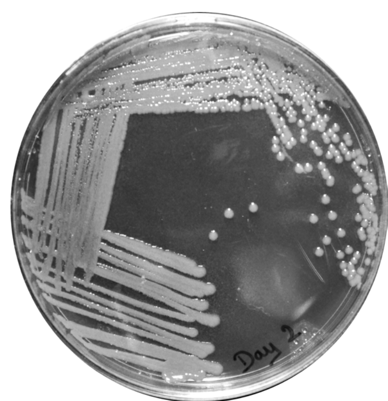

Fig. 1. Typical red pigment colonies of *Serratia marcescens* (NPSM-1) isolated from fin rot lesions in guppy, *Poecilia reticulata*

pigmented bacteria revealed that it is an enterobacteria belonging to the species *S. marcescens* (NPSM-1).

According to Austin and Austin (2007), bacteria such as *Aeromonas* sp., *Bacillus* sp., *Citrobacter* sp., *Edwardsiella* sp., *Flavobacterium* sp., *Klebsiella* sp., *Proteus* sp., *Providencia* sp. and *Serratia* sp, are associated with fish disease and several bacterial pathogens have been isolated from freshwater fish by various workers (Kumar and Dey, 1988; Das *et al*., 1999; Novotny *et al*., 2004; Mohanty and Sahoo, 2007) in India. Recently Kumar *et al*. (2015) isolated a zoonotically important bacterial pathogen; *Proteus hauseri* causing mass mortality of ornamental koi carp in India. *S. marcescens* was isolated and confirmed only by biochemical characteristics from marine ornamental fish with ulcerative disease in India (Pramila, 2002). In the present study *S. marcenscens* was isolated and identified from diseased guppy fish *P. reticulata*.

Sequence analysis of 16S rRNA, metalloprotease and quorum sensing genes of S. marcescens

Approximately 1470 bp gene sequence of 16S rRNA was amplified after assembling the forward and reverse sequences. The 1408 bp and 1486 bp of metalloprotease gene (*Pr596*) and AHL synthase gene (*Spn*I) were also amplified and sequenced (Fig. 2). The BLAST results of 16S rRNA gene, *Pr596* and *Spn*I showed that the isolate shared 99.5, 98 and 99% similarity respectively with *S. marcescens*. This is the first description of *S. marcescens* strains as pathogens of guppy *Poecilia reticulata*. To date, only limited scientific reports on *Serratia* sp. associated fish diseases are documented. Nieto *et al*. (1990) isolated *S. plymuthica* from moribund rainbow trout in north-western Spain, McIntosh and Austin (1990) isolated bacteria resembling *S. liquefaciens* from salmonids in Australia and Scotland and Baya *et al*. (1992) isolated

Table 1. Biochemical characteristics of *Serratia marcescens* (NPSM-1) bacterial strains isolated from guppy mass mortality in this study

Biochemical tests	*S. marcescens* (NPSM-1) isolated in this study	*S. marcescens* ATCC 8100	*S. marcescens* isolated from experimentally challenged fish
Gram stain	-	-	-
Motility	+	+	+
Oxidase	-	-	-
Catalase	+	+	+
Oxidative/ Fermentative glucose	F	F	F
Methyl red	-	-	-
Voges-Proskauer	+	+	+
Indole production	-	-	-
Nitrate reduction	+	+	+
Citrate utilisation	+	-	+
Growth at 15^0C	+	+	+
Growth at 25^0C	+	+	+
Growth at 37 ^{0}C	+	+	+
Growth at 0% NaCl	+	+	+
Growth at 3% NaCl	+	+	+
Growth at 6% NaCl	+	+	+
Arginine decarboxylase	-	-	-
Lysine decarboxylase	+	+	+
Ornithine decarboxylase	+	-	+
Haemolysis on sheep blood agar	*β*	*β*	*β*
Sugar utilisation			
Mannose	+	+	+
Galactose	+	+	+
Fructose	+	+	+
Maltose	+	+	+
Sucrose	+	+	+
Rahmnose	-	-	-
Arabinose	-	-	-
Salicin	+	+	+
Trehalose	+	+	+
Lactose	-	-	-
Xylose	-	-	-
Cellobiose	-	-	-
Raffinose	-	-	-
Mannitol	+	+	+
Sorbitol	+	+	+

S. marcescens from white perch, *Morone americanus* in USA. Although the origin of the *S. marcescens* in the diseased guppy is not known, it is possible that the bacteria may have originated from one of the farm personnel in close contact with the fish. *S. marcescens* is a well known human pathogen causing respiratory tract and urinary tract infection as well as endocarditis, osteomyelitis, pneumonia and meningitis (Hejazi and Falkiner, 1997).

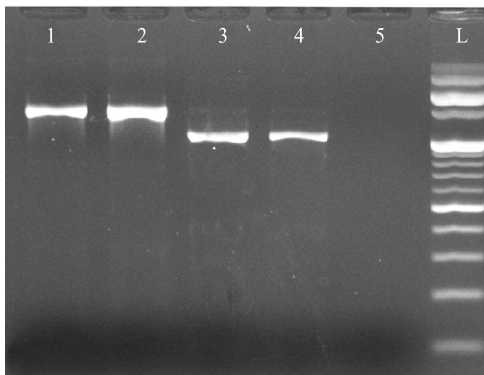

Fig. 2. Agarose gel image showing PCR-amplified 1408 bp and 1486 bp bands of metalloprotease gene (*Pr596*) from *Serratia marcescens* (NPSM-1).
Lanes 1 and 2 - metalloprotease gene (*Pr596*); Lanes 3 and 4 - AHL synthase gene (*SpnI*); Lane 5 - DNA template from *Aeromonas hydrophila* as negative control. Lane L: Low range DNA ruler, 100 bp to 3 kbp (GeNei)

Extracellular metalloproteases, *Pr596* are mostly associated with pathogenic bacteria or bacteria that have industrial significance (Hase and Finkelstein, 1993). Many Gram negative pathogens control the expression of virulence factors, secretion of extracellular protease, pectinase and rhamnolipid and biofilm formation *via* the quorum-sensing system (de Kievit and Iglewski, 2000). The regulation of flagellum independent populational surface migration, the synthesis of biosurfactant and production of prodigiosin and nuclease in *S. marcescens* SS-1 are co-ordinately negatively monitored by *spnI*R (Horng *et al*., 2002). It has been shown that *Serratia* strains employ quorum sensing for the regulation of genes encoding extracellular virulence factors. In *Serratia*, at least four different LuxRI/AHL quorum sensing systems *viz., Spr*IR from *Serratia proteamaculans*, *Swr*IR from *Serratia liquefaciens* MG1 (now renamed as *S. marcescens* MG-1) *Spn*IR from *S. marcescens* SS-1 and *Sma*IR from *Serratia* sp. ATCC39006 have been described (Wei and Lai, 2006).

Phylogenetic tree

The phylogenetic tree was constructed based on 1450 bp of 16S rRNA gene sequences (Fig. 3). The 16S rRNA sequence of NPSM-1 was compared with published sequences of *S. marcescens* and sequences of other species *viz., S. liquefaciens, S. plymuthica, S. odorifera, S. glossinae* and *S. ficania.* Distantly related bacteria *V. cholerae was* taken as outgroup. It generated clusters, which were supported by bootstrap values of 93 and 100.

Haemolytic assay

S. marcescens produces a range of secreted products, including lipases, proteases, chitinases, nucleases, biosurfactants and haemolysin (Hejazi and Falkiner, 1997). In our study, when *S. marcescens* colonies were spotted on 10% sheep blood agar, they did not form haemolysis zones at 24 h of incubation at 28°C, but after incubation for an additional 12 h at 4°C the haemolytic zones were observed (Fig. 4). Haemolysin production is a common attribute of *S. marcescens* strains and has been shown to be involved in the virulence of this pathogen

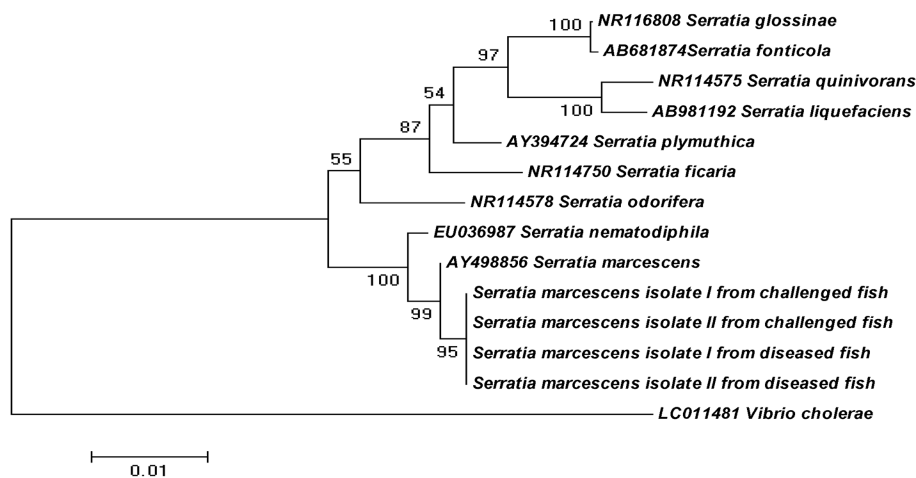

Fig. 3. Phylogenetic tree based on the concatenated sequences of 16S rRNA of *Serratia marcescens* (NPSM-1) isolated from guppy and related *Serratia* species. The neighbour-joining algorithm was used to construct the tree with genetic distance computed by Kimura's 2-parameter method. Bootstrap values of 1000 simulations are indicated at the branches. The bar indicates percentage difference

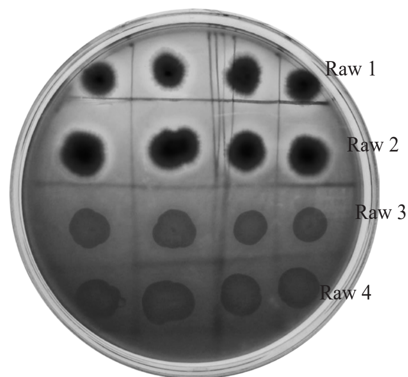

Fig. 4. Haemolysis of sheep erythrocytes on 10% sheep blood agar plates after 24 h of incubation at 28°C and further incubation at 4°C for 12 h.
Row 1 and 2 - isolates of *Serratia marcescens* (NPSM-1) showing swarming growth and zones of haemolysis, Row 3 and 4 - colonies of non-haemolytic *Aeromonas* sp.

(Hilger and Braun, 1995). *S. marcescens* secretes a haemolysin that also acts as a cytotoxin and the haemolytic activity is determined by the *shl*A and *shl*B genes (Poole *et al*., 1988). In the absence of *Shl*B, inactive *Shl*A of *S. marcescens* remains in the periplasm and displays less haemolytic activity with small haemolysis zone (Schiebel *et al*., 1989).

Cytotoxicity

The ECP from the NPSM-1displayed a positive cytotoxic response on the CCKF cell line tested within 24 h. The cytotoxic changes started appearing in the CCKF cells within 6 h post-inoculation (hpi) and followed by lysis of cells after 24 hpi. No morphological changes could be detected in the control cells inoculated with PBS. Cytotoxic changes *viz.,* granulation, vacuolation, rounding and dislodgement of cells, were recorded on microscopic examination of the infected CCKF cell line (Fig. 5a, b, c and d). Vacuoles in different epithelial cell lines *viz.,* adherent HEp-2, RT112, HeLa, Chang and Hec1B cells were observed when treated with supernatant culture of *S. marcescens* within 15 min, followed by lysis after 40 min (Hertle *et al*., 1999). A similar cytotoxic change to that of *Shl*A - induced vacuolation has been observed with aerolysin from *Aeromonas hydrophila* (Abrami *et al*., 1998). *S. marcescens* exerts haemolytic and vacuolating cytotoxic activities mainly in direct contact with the different target cells (Braun *et al*., 1985).

Antibiotic sensitivity

The susceptibility of *S. marcescens* to antibacterial agents varied. The bacteria was resistant to ampicillin, amoxicillin, cefalaxein, furazolidone, kanamycin, nitrofuratoin and oxtetracycline while susceptible to cefixime, chloramphenicol, ciprofloxacin, erythromycin and gentamycin. Similar observations concerning *S. marcescens* isolated from clinical cases of marine and freshwater fish were also previously described (Baya *et al*., 1992). Resistance of *S. marcescens* to 11 out of 16 tested antimicrobial agents was observed. This suggests development of multidrug resistance by this bacteria and need for identifying appropriate antibiotic treatment against this bacterium, supported by antibiotic susceptibility testing. The fish farmer was advised to administer ciprofloxacin in the feed to affected fish at the dose rate of 5 mg g^{-1} feed twice daily for five days. After administering the antibiotic, fish recovered from the disease and no further mortality of guppy was noticed in the farm.

Experimental challenge tests

Fish infected with NPSM-1showed the following external signs *viz.,* dark skin, necrotic skin lesions and fin rot. *S. marcescens* was reisolated in pure cultures from infected fish. No mortality or any disease signs were observed in fish injected with sterile PBS. The mortality

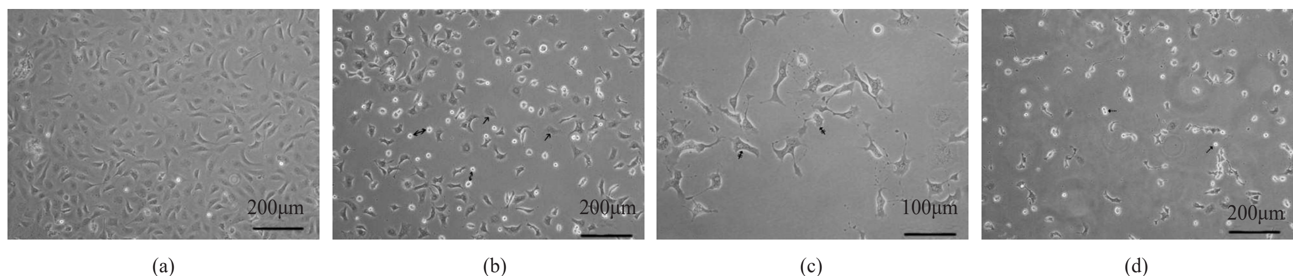

Fig. 5. Phase-contrast photomicrographs of *Serratia marcescens* (NPSM-1) ECP-induced cytotoxic changes of CCKF cells. (a) Control; (b) 6 h post inoculation (hpi); (c), 12 hpi; (d) 24 hpi. After incubation at 28°C, cells were examined by phasecontrast microscopy. Cytotoxicity changes *viz.,* granulation (← - arrow), vacuolation (‡ - crossed arrow), rounding (↔ - double sided arrow) and dislodgement (⇢ - dotted arrow) of CCKF cells were recorded subsequent to the addition of ECPs of *S. marcescens* (NPSM-1)

rate of the experimentally challenged group was 55% as reported in earlier studies (Baya *et al*., 1992). In this study, the reisolation of *S. marcescens* from freshly dead experimental fish and its confirmation by sequencing fulfilled Koch's postulates. The results suggest that the present *S. marcescens* strain could be considered a potential bacterial pathogen for fish.

It has been demonstrated (Buras *et al*., 1985) that a high bacterial load in water, stresses the fish immune system and result in invasion and proliferation of environmental bacteria in the fish tissues. There is a possibility for dissemination of *S. marcescens* to other geographic areas through the water and fish trade. Chan *et al*. (2013) isolated *S. marcescens* W2.3, a suspected causal agent of an endemic disease outbreak along with other bacteria from the tilapia fish farms of Malaysia during 2009. The emergence of multidrug resistant *Serratia* sp., has been distressing in the human medical field and also in aquaculture and agriculture sectors (Kurz *et al*., 2003). In addition, the potential pathogenic capability and multidrug resistance of this isolate may be of public health concern since *S. marcescens* is a well recognised opportunistic pathogen causing important human infections.

The isolation of the highly virulent and multidrug resistant zoonotically important bacterium *S. marcescens*, from freshwater ornamental fish in India poses a threat that this pathogen could cause infections to the farm personnel. This may be given due attention as they might cause zoonotic diseases. Further epidemiological investigations together with studies on *S. marcescens* pathogenicity are necessary to elucidate the public health significance of *S. marcescens*.

Acknowledgements

The authors are thankful to the Secretary, Department of Agricultural Research and Education and Director General, Indian Council of Agricultural Research and DDG (Fy) ICAR, New Delhi, India for their support, encouragement and guidance. We thank National Fishery Development Board, Ministry of Agriculture. Government of India for their financial support.

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Date of Receipt : 20.06.2017
Date of Acceptance : 06.10.2017