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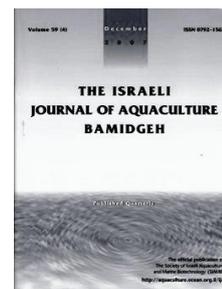
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***Citrobacter freundii*: a causative agent for ulcer disease in snakehead fish *Ophiocephalus argus* (Cantor)**

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Keywords: ulcer disease; *Ophiocephalus argus* (Cantor); *Citrobacter freundii*; antibiotic resistance.

Abstract

Ulcer disease has caused significant economic losses in the culture of snakehead fish *Ophiocephalus argus* (Cantor). Information available on *Citrobacter freundii* as a possible causal agent for this disease is scarce. In this study, a virulent strain, temporarily named GB1, was isolated from diseased snakehead fish suffering from ulcer disease and was identified as *C. freundii* through molecular and phenotypic methods. A phylogenetic tree was constructed to examine isolate GB1 and compare it to other known isolates. In addition, isolate GB1 has developed resistance to aminoglycosides, tetracyclines, and sulfonamides drugs for veterinary use in aquaculture as revealed when screened against a range of common antibiotics. To the best of our knowledge, this is the first report of . ulcer disease caused by *C. freundii* in snakehead fish

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Introduction

Snakehead fish *Ophiocephalus argus* (Cantor) is widely cultivated in many countries such as China, India, Korea, Malaysia, Philippines, and Thailand (Lee et al., 1993; Tortoli et al., 1996; Dhanaraj et al., 2008; Saikia et al., 2018; Samayanpaulraj et al., 2019). In China in particular, with the rapid development of farming techniques, snakehead fish has become one of the most important commercial freshwater fish species and has been very profitable in recent years (Sagada et al., 2017). Production increased to over 483,000 tons in 2017 (Ministry of Agriculture and Rural Affairs of China, 2018). However, under intensive culture, this industry has been seriously affected by bacterial diseases (Chen et al., 2012; Sundberg et al., 2016). Thus, more attention should be paid to bacteriosis to enable further development of this industry.

Ulcer disease is one of the most important infectious bacterial diseases in snakehead fish (Dhanaraj et al., 2008), causing high mortality of over 90% (Wang et al., 2017). Several bacterial pathogens such as *Aeromonas veronii* (Lee et al., 1993), *Aeromonas hydrophila* (Yu et al., 2014) and *Aeromonas sobria* (Jin et al., 2016) have been reported to cause this disease. However, little information is available on *Citrobacter freundii* as a causal agent for ulcer disease in snakehead fish.

In the present study, *C. freundii* was isolated from snakehead fish suffering from ulcer disease in Guangdong China during April 2018. The aim of this study was to characterize the phenotype, taxonomic position, and antibiotic sensitivity of this strain. As far as we know, this is the first report of *C. freundii* as a pathogen of ulcer disease in snakehead fish.

Materials and methods

Snakehead fish samples

Nineteen ulcer disease-infected snakehead fish (average weight 500±15 g) were sampled from a fish farm in Guangdong China during April 2018. The outbreak of this disease occurred in 6,000 square meters of ponds with snakehead fish at the initial stocking density of 300 juveniles per square meter and resulted in a high cumulative mortality of over 80% despite the use of glutaraldehyde. Water quality during the disease outbreak was pH 8, 0.40 mg/L, total ammonia, 0.15 mg/L nitrite, and 0.40 mg/L dissolved oxygen. Diseased samples were placed in sterile bags, kept in ice, and transported to the laboratory.

Isolation of Bacteria

Each sampled diseased snakehead fish was externally disinfected with 75% alcohol and dissected. 0.1 g of ulcerative muscle and liver sample of each diseased fish was cut and streaked onto nutrient agar (NA) plates and Thiosulfate citrate bile salts sucrose agar (TCBS) plates (Sinopharm Chemical Reagent Co., Ltd.). After incubation for 24h at 28°C, the dominant uniform isolates were purified by streaking and re-streaking onto NA plates. Pure isolates of the dominant colonies were stored at -80°C supplemented with 15% glycerol. A representative of the dominant isolates, temporarily named GB1, was characterized further in the present study.

Identification of the pathogen

Molecular identification

The extraction of genomic DNA from isolate GB1, as well as PCR amplification and sequencing of its 16S rRNA gene were performed according to our previous study (Cao et al., 2010). The near complete 16S rRNA gene sequence was assembled using MegAlign, Editseq and Seqman software. A search was performed in the National Centre for Biotechnology Information (NCBI) database for sequence homology using the Basic Local

Alignment Search Tool (BLAST) program. A phylogenetic tree from the near complete 16S rRNA gene sequence of the isolate and its homologous sequences was constructed using the neighbor-joining method.

Phenotypic identification

Isolate GB1 was identified phenotypically by API 20E system recommended by Topic Popovic et al. (2007) where the isolate GB1 was grown on nutrient agar (NA) plates (Sinopharm Chemical Reagent Co., Ltd.) at 28°C for 24h, and the bacterial suspension was then used to inoculate the Analytical Profile Index (API 20E) test strips (Biomerieux, France) following the manufacturer's instruction. The plate was incubated at 37°C and observed after 18h for checking against the API identification index and database. Information related to *C. freundii* previously reported by Dong & Cai (2001) and Shen et al. (2005) served as a reference.

Bacterial virulence assay

Bacterial virulence was examined by experimentally infecting healthy snakehead fish. One hundred healthy snakehead fish average weight 62.5 ± 2.5 g, were obtained from a snakehead fish farm in Jiangsu China. The experimental fish were acclimated in ten aquaria (ten fish per aquarium) supplied with 100 L of aerated filtered farming water at 28°C for 14 days. Prior to the bacterial virulence assay the isolate GB1 was inoculated onto NA plate, incubated at 28°C for 24h, then washed with normal saline into a sterile tube. Cell density was determined by counting colony forming units after a ten-fold serial dilution in sterile distilled water. Two replicates of ten healthy fish were challenged by intramuscular injection (Zhang et al., 2012; Xiao et al., 2016) with 0.1 mL of the isolate GB1 at a concentration of 2.0×10^4 CFU/mL to 2.0×10^7 CFU/mL. Another two replicates of ten healthy fish exposed to the same experimental conditions and injected intramuscularly 0.1 mL of normal saline remained unchallenged and served as the control. The experimental fish were kept at 28°C and observed daily for seven days without feeding and water change. Any dead fish were immediately removed and sampled to re-isolate and confirm if mortality was caused specifically by the challenge isolate. The mean lethal dose (LD₅₀) value was calculated using the linear regression method as recommended by Spielmann et al. (1999).

Antibiotic sensitivity assay

Antibiotic sensitivity of isolate GB1 was assayed on NA plates using the Kirby-Bauer disk diffusion method as recommended by Jones et al. (2001). Sixteen fishery antibiotic discs were acquired from Hangzhou Binhe Microorganism Reagent Co., Ltd. The zones of inhibition were measured after a 24h incubation period at 28°C. Antibiotic susceptibility was determined according to the manufacturer's guidelines.

Results

Identification of the pathogenic isolate

A dominant isolate GB1 was isolated from the diseased snakehead fish and identified by molecular and phenotypic methods as *C. freundii*. Its near complete 16S rRNA gene sequence (1400 nucleotides) was submitted to GenBank database with the accession no. MK806489. A similarity of 99% to 100% was observed in the 16S rRNA gene sequence between the GB1 isolate and other *C. freundii* isolates from the GenBank database. The phylogenetic tree confirms that the isolate GB1 is identified with *C. freundii* strain (Figure 1). This is again confirmed by the phenotypic features as *C. freundii* (Table 1) with 100% identity compared to the reference strain.

Figure 1. A 16S rRNA gene tree of 13 known bacteria and the GB1 isolate constructed using the neighbor-joining method. The bootstrap values (%) are shown besides the clades, accession numbers are indicated beside the names of strains, and scale bars represent distance values.

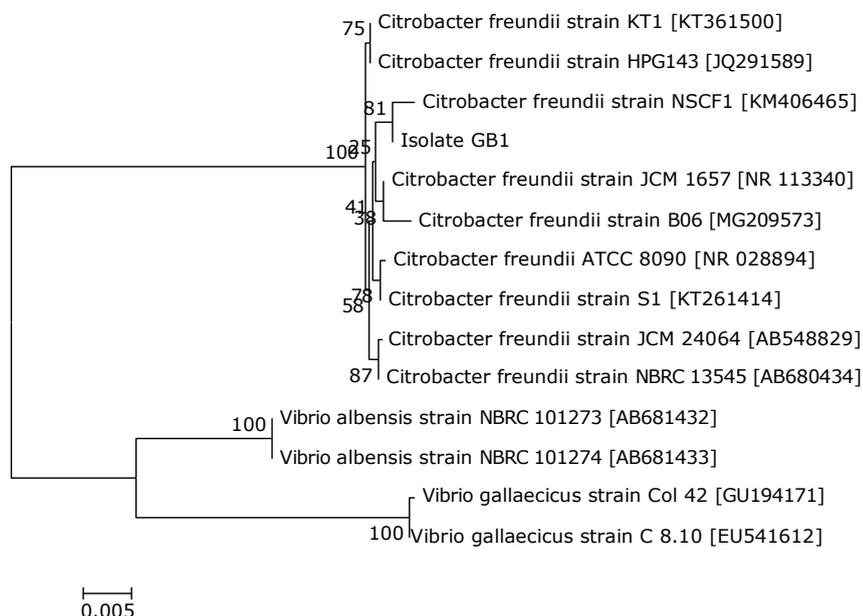


Table 1. Phenotypic characterization of isolate GB1.

Tests	Reaction	
	Isolate GB1	<i>C. freundii</i> ^a
Arginine dihydrolase	R ⁻	R ⁻
Cytochrome oxidase	R ⁻	R ⁻
β-Galactosidase	R ⁺	R ⁺
Gelatinase	R ⁻	R ⁻
Lysine decarboxylase	R ⁻	R ⁻
Ornithine decarboxylase	R ⁻	R ⁻
Tryptophan deaminase	R ⁻	R ⁻
Urease	R ⁻	R ⁻
Citrate utilization	R ⁻	R ⁻
Acetoin production	R ⁻	R ⁻
Indole production	R ⁻	R ⁻
H ₂ S production	R ⁺	R ⁺
Arabinose fermentation	R ⁺	R ⁺
Amygdalin fermentation	R ⁻	R ⁻
Glucose fermentation	R ⁺	R ⁺
Inositol fermentation	R ⁻	R ⁻
Mannitol fermentation	R ⁺	R ⁺
Melibiose fermentation	R ⁺	R ⁺
Rhamnose fermentation	R ⁺	R ⁺
Sucrose fermentation	R ⁺	R ⁺
Sorbitol fermentation	R ⁺	R ⁺

R⁺: positive reaction; R⁻: negative reaction.

^aThe reference strain data are in accordance with those previously reported (Dong & Cai, 2001; Shen et al., 2005).

Isolate GB1 was found to be pathogenic in an experimental challenge. The death rate of the experimental fish increased gradually after the challenge. 20%-100% of the snakeheads challenged with isolate GB1 died at a LD₅₀ value of 2.61 × 10⁵ CFU/mL (Table 2) and exhibited rot and necrosis of muscles, similar to that seen in the originally

diseased snakehead fish (Figure 2). In addition, the re-isolated bacteria from experimentally dead fish are identified phenotypically and molecularly as isolate GB1. No clinical signs or mortality were noted in the control snakehead fish.

Table 2. Cumulative mortality of experimental snakehead fish infected by isolate GB1.

Group	Concentration (CFU/mL)	Fish no.	Dead fish no. on day after challenge							Average cumulative mortality (%)	LD ₅₀ value (CFU/mL)
			1	2	3	4	5	6	7		
Control	0	10	0	0	0	0	0	0	0	0	2.61×10 ⁵
		10	0	0	0	0	0	0	0		
Treatment 1	2.0 ×10 ⁴	10	0	0	1	1	0	0	0	20	
		10	0	1	0	1	0	0	0		
Treatment 2	2.0 ×10 ⁵	10	0	1	1	1	0	0	0	30	
		10	1	0	1	1	0	0	0		
Treatment 3	2.0 ×10 ⁶	10	2	3	1	1	1	0	0	85	
		10	1	2	2	2	1	1	0		
Treatment 4	2.0 ×10 ⁷	10	6	3	1	0	0	0	0	100	
		10	5	4	1	0	0	0	0		

Figure 2. Pathological symptoms of the snakehead fish suffering from ulcer disease. Arrow shows the rotten and necrotic muscle.



Antibiotic sensitivity

The antibiotic sensitivity of isolate GB1 is shown in Table 3.

Table 3. Susceptibility of isolate GB1 to antibiotics.

Antibiotics	Content (µg/disc)	Inhibition zone diameter (mm)
Amoxicillin	10	0±0 ^R
Ampicillin	10	0±0 ^R
Cefotaxime	30	21.50±0.5 ^I
Cotrimoxazole*	23.75/1.25	0±0 ^R
Doxycycline*	30	0±0 ^R
Enrofloxacin*	5	21.50±0.5 ^S
Erythromycin	15	8.50±0.25 ^R
furazolidone	30	8.30±0.20 ^R
Gentamicin	10	0±0 ^R
Kanamycin	30	0±0 ^R
Neomycin*	30	8.25±0.25 ^R
Norfloxacin*	10	21.5±0.25 ^S
Ofloxacin	5	25.75±0.25 ^S
Penicillin	10	0±0 ^R
Sulfamethoxydiazine*	5	0±0 ^R
Tetracycline	30	7.25±0.25 ^R

Data are presented as the mean ± standard deviation; ^SSensitive; ^IIntermediately sensitive; ^RResistant. *Antibiotics for aquaculture use (Ministry of Agriculture of China, 2013).

The data indicate that isolate GB1 is sensitive to enrofloxacin, norfloxacin, ofloxacin, intermediately sensitive to cefotaxime, and resistant to amoxicillin, ampicillin, cotrimoxazole, doxycycline, erythromycin, furazolidone, gentamycin, kanamycin, neomycin, penicillin, sulfamethoxydiazine, tetracycline. This suggests that isolate GB1 has developed resistance to aminoglycosides, tetracyclines, and sulfonamides drugs used in aquaculture.

Discussion

In aquaculture, the association of *C. freundii* with massive mortality reported in *Mola mola* (Sato., 1982), *Eriocheir sinensis* (Li et al., 2001), *Oreochromis niloticus* (Hu et al., 2014), *Andrias davidianus* (Gao et al., 2012), *Procambarus clarkii* (Chen et al., 2014; Xiao et al., 2016), *Pelodiscus sinensis* (Mao et al., 2015) and *Macrobrachium rosenbergii* (Feng et al., 2017) has been well documented. However, there is limited information on *C. freundii* isolates as causal agents for ulcer disease in snakehead fish. In this study, we characterized the phenotype, taxonomic position, and antibiotic susceptibility of *C. freundii* GB1. To our knowledge, this is the first report of a *C. freundii* pathogen as a causative agent for ulcer disease in snakehead fish.

Citrobacter are species with virulence factors including the ability to survive the serum bactericidal activity, the resistance to the intracellular killing in the polymorphonuclear leucocytes and the cell surface hydrophobicity (Nayar et al., 2013). Diseases caused by *C. freundii* are usually associated with the production of these virulent factors. In the present study, the GB1 isolate was found to cause mortality in healthy snakehead fish with a LD₅₀ value of 2.61×10^5 CFU/mL. This further demonstrates the potential threat of *C. freundii* to freshwater farming of snakehead fish. Apart from the virulence of the GB1 isolate, there might be other secondary factors that induce ulcer disease in snakehead fish such as over intensification of stocking density, poor health status, use of contaminated feed, and lack of effective water disinfection (Yu et al., 2014); these should also be raised as concerns.

Antibiotic resistance in *C. freundii* has been reported in aquaculture as a result of the wide use of antibiotics (Cao et al., 2016). For example, a *C. freundii* isolate from diseased *A. marmorata* has been found to be resistant to cotrimoxazole and neomycin (Yang et al., 2013), and a *C. freundii* isolate from diseased *M. amblycephala* has been documented to develop resistance against cotrimoxazole and doxycycline (Zhang et al., 2016). The GB1 isolate in our study also developed resistance to multiple antibiotics including cotrimoxazole, doxycycline, neomycin, and sulfamethoxydiazine used in fish farming regions, suggesting that the outbreak of this disease may have resulted from the abuse of antibiotics.

In conclusion, for the first time, the present study reports a *C. freundii* isolate as a causal agent for ulcer disease in snakehead fish. The pathogenicity and multiple drug resistance of the GB1 isolate support the claim that this infection is an emerging threat in snakehead fish farming.

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References

- Cao H., He S., Lu L., Hou L.**, 2010. Characterization and phylogenetic analysis of the bitrichous pathogenic *Aeromonas hydrophila* isolated from diseased Siberian sturgeon. *Israeli J Aquacult-Bamidgeh*, 3: 182-189. http://cmsadmin.atp.co.il/Content_siamb/editor/62_3_7_Cao.pdf
- Cao H., Long X., Lu L., Yang X., Chen B.**, 2016. *Citrobacter freundii*: a causative agent for tail rot disease in freshwater cultured Japanese eel *Anguilla japonica*. *Isr. J. Aquacult.-Bamidgeh, AquacultureHub*, 68.2016.1271.
- Chen H., Song G., He J., Hou G., Wang Y.**, 2014. Isolation, identification and antibiotics susceptibility test of *Citrobacter freundii* from *Procambarus clarkii*. *Freshwater Fisheries*, 44(01):73-77.
- Chen Y., Liang R., Zhuo X., Wu X., Zou J.**, 2012. Isolation and characterization of *Aeromonas schubertii* from diseased snakehead, *Channa maculata* (Lacepède). *J Fish Dis*, 35(6): 421-430.
- Dhanaraj M., Haniffa M. A. K., Ramakrishnan C. M., Singh S. V.**, 2008. Microbial flora from the epizootic ulcerative syndrome (EUS) infected murrel *Channa striatus* (Bloch, 1797) in Tirunelveli region. *Turkish J Vet Anim Sci*, 32(03): 221-224.
- Dong X.Z., Cai M.Y.** 2001. *General manual of systematic and determinative bacteriology*. Science Press, Beijing, 76pp.
- Feng Y., Yang S., Yang Y., Wu Y., Chen J.**, 2017. Isolation and identification of *Citrobacter freundii* from diseased giant freshwater prawn (*Macrobrachium rosenbergii*). *Chinese Vet Sci*, 47(11): 1411-1417.
- Gao Z., Zeng L., Meng Y., Liu X., Zhang B.**, 2012. Isolation and identification of *Citrobacter freundii* from diseased giant salamander *Andrias davidianus*. *Acta Microbiologia Sinica*, 52(2): 167-176.
- Hu X., Lv A., Liu Y., Wang Y.**, 2014. Isolation and identification of *Citrobacter freundii* from the intestine of tilapia. *Heilongjiang Animal Sci Vet Med*, (21): 174-176.
- Jin H., Song C., Wang L., Li J., Huang X., Zhang X.**, 2016. Isolation and identification of *Aeromonas sobria* S8 from hybrid snakehead (*Channa maculate* ♀ × *Channa argus* ♂) with ulcerative disease and screening of Chinese herbal medicine for prevention and treatment. *Freshwater Fisheries*, 46(5): 49-54.
- Jones R.N., Ballou C.H., Biedenbach D.J.**, 2001. Multi-laboratory assessment of the linezolid spectrum of activity using the Kirby-Bauer disk diffusion method: Report of the Zyvox® antimicrobial potency study (ZAPS) in the United States. *Diag Microbiol Infect Dis*, 40: 59-66.
- Lee H.K., Lee T.Y., Kim B.S.** 1993. Histopathogenic characteristics of haemorrhagic ulcer in cultivated snakehead (*Channa argus*) artificially infected with *Aeromonas veronii*. *Korean J Microbiol*, 31(2): 113-122.
- Li H., Xing D., Bai G., Li X.**, 2001. Studies on disease of mitten crab caused by *Citrobacter freudii*. *Acta Hydrobiologica Sinica*, 25(3):217-223.
- Mao S., Si G., Ren X., Shi T., Zhang Y., Wang Z., Zhang X.**, 2015. Isolation and identification of multidrug resistant *Citrobacter freundii* from diseased soft-shelled turtle *Pelodiscus sinensis*. *Freshwater Fisheries*, 45(2): 102-106.
- Ministry of Agriculture of China**, 2013. *The First Catalogue of Veterinary Prescription Drugs*. *China Animal Health*, 15(11): 81-83.
- Ministry of Agriculture and Rural Affairs of China**, 2018. *China Fishery Statistical Yearbook*. China Agriculture Press, Beijing, 25pp.

- Nayar R., Shukla I., Ali S.M.,** 2013. Evaluation of the virulence markers in clinical isolates of *Citrobacter* species: the first report from India. *J Clin Diag Res*, 7(6): 1031-1034.
- Sagada G., Chen J., Shen B., Huang A., Sun L., Jiang J., Jin C.,** 2017. Optimizing protein and lipid levels in practical diet for juvenile northern snakehead fish(*Channa argus*). *Anim Nutr*, 3(2): 156-163.
- Saikia D.J., Chattopadhyay P., Banerjee G., Talukdar B., Sarma D.,** 2018. Identification and pathogenicity of *Pseudomonas aeruginosa* DJ1990 on tail and fin rot disease in spotted snakehead. *J World Aquacult Soc*, 49(4): 703-714.
- Samayanpaulraj V., Velu V., Uthandakalaipandiyar R.,** 2019. Determination of lethal dose of *Aeromonas hydrophila* Ah17 strain in snakehead fish *Channa striata*. *Microbial Pathogenesis*, 127: 7-11.
- Sato N., Yamane N., Kawamura T.,** 1982. Systemic *Citrobacter freundii* infection among sunfish *Mola mola* in Matsushima Aquarium. *Bulletin of the Japanese Soc Sci Fisheries*, 48(11): 1551-1557.
- Shen J., Gu Z., Pan X., Zhou B., Yi W., Cao Z.,** 2005. Isolation and identification of *Citrobacter freundii* from *Cherax quadricarinatus*. *J Fish Sci China*, 12(2): 197-200.
- Spielmann H., Genschow E., Liebsch M., Halle W.,** 1999. Determination of the starting dose for acute oral toxicity (LD50) testing in the up and down procedure (UDP) from cytotoxicity data. *Alternatives to Laboratory Animals*, 27: 957-966.
- Sundberg L.R., Ketola T., Laanto E., Kinnula H., Bamford J.K.H., Penttinen R., Mappes J.,** 2016. Intensive aquaculture selects for increased virulence and interference competition in bacteria. *Proceedings of the Royal Society B*, 283: 1-10.
- Tortoli E., Bartoloni A., Bozzetta E., Burrini C., Lacchini C., Mantella A., Penati V., Simonetti M. T., Ghittino C.,** 1996. Identification of the newly described *Mycobacterium poriferae* from tuberculous lesions of snakehead fish (*Channa striatus*). *Comp Immunol, Microbiol Infect Dis*. 19(1): 25-29
- Topic Popovic N., Coz-Rakovac R., Strunjak-Perovic I.,** 2007. Commercial phenotypic tests (API 20E) in a diagnosis of fish bacteria: a review. *Veterinarni Medicina*, 52(2): 49-53.
- Wang Y., Cheng Y.,** 2017. Etiology and control measures of ulcer disease in snakehead fish. *Sci Fish Farming*, 5:64-65.
- Xiao N., Kong L., Zhou H., Cao J., Li J., Xiang Y., Wang M.,** 2016. Isolation and identification of *Citrobacter freundii* from diseased *Procambarus clarkii* and its antibiotics susceptibility and adhesion characteristics. *J Fish China*, 40(06): 946-955.
- Yang F., Guan R., Li Z., Yu H., Li Q.,** 2013. Identification of pathogenic *Citrobacter freundii* isolated from *Anguilla marmorata*. *J Jimei Univ (Natural Science)*, 18(02): 81-87.
- Yu Y., Wang X., Peng C., Zhao Q., Jin S.,** 2014. Pathogenic and hemopathological studies on the skin-ulcerating disease of *Channa argus*. *Freshwater Fisheries*, 44(04): 73-76.
- Zhang D., Kang Y., Tian J., Chen H., An D., Xu R., Sun W., Shan X., Qian A.,** 2016. Isolation, identification and drug susceptibility of the pathogenic *Citrobacter freundii* from *Megalobrama amblycephala*. *Chinese Vet Sci*, 46(12): 1589-1595.
- Zhang J., Pan L.,** 2012. Identification of the pathogen isolated from the skin-ulcerated *Acanthurus glaucopareius*. *J Shanghai Ocean University*, 21(01): 48-53.