Isolation of *Photobacterium damselae* subsp. *piscicida* from diseased tongue sole (*Cynoglossus semilaevis* Gunther) in China

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Abstract: During the summer of 2006, an epizootic occurred among cultured tongue sole (*Cynoglossus semilaevis* Gunther) in a fish farm in Qingdao, China. The diseased tongue sole exhibited haemorrhaging of the basal fin, yellowed kidney and ulceration of the body surface. A Gram-negative, rod shaped bacterium (designated strain WY06) was isolated from the gall bladder of diseased fish. Pathogenicity assays revealed that WY06 was virulent to tongue sole and zebrafish (*Danio rerio*) by intraperitoneal injection challenge, with the LD_{50} being calculated as 5.5×10^3 cfu/g of fish (5.2×10^5 cfu/fish) and 1.9×10^3 cfu/g of fish (8.9×10^2 cfu/fish) respectively. The 16S rRNA gene sequence of strain WY06 demonstrated high similarity (99%) with *Photobacterium damselae* subsp. *piscicida*. Phylogenetic analysis showed a clear association of strain WY06 with *P. damselae* subsp. *piscicida*. Additional evidence of the identification included in morphological, physiological and biochemical data. The pathogen was sensitive to cifuroxime (30μg) and ceftriaxone sodium (30μg). Present study describes *P. damselae* subsp. *piscicida* from diseased fish for the first time in China.

Keywords: *Photobacterium damselae* subsp. *piscicida*; pasteurellosis; *Cynoglossus semilaevis* Gunther; pathogenicity; 16S rDNA
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1 INTRODUCTION

Photobacterium damselae subsp. *piscicida* is the cause of pasteurellosis, which was first described in wild populations of white perch (*Morone americanus*) and striped bass (*Morone saxatilis*) in 1963 in Chesapeake Bay, USA[1]. At the end of the sixties and the beginning of the seventies of 20th century, the bacterium for the first time became economically important causing severe problems, and high mortalities were reported in Japanese aquaculture[2]. In Europe, Toranzo, *et al*.[3] described the first outbreak of pasteurellosis in Spain in 1990, which affected juvenile gilthead seabream (*Sparus aurata*) cultures in the northwestern area. Soon thereafter, it caused economic losses in the marine culture of gilthead seabream, seabass and sole (*Solea senegalensis* and *Solea solea*) in the Mediterranean countries of Europe and other coutries[3~9]. Pasteurellosis continues to be a severe problem in intensive culture of different fish species in the Mediterranean and Japan. Until recently, China was considered to be free of fish pasteurellosis.

Tongue sole is an economically important marine fish species, and is valued for its rapid growth and good taste. Over the years, there has been a steady demand for tongue sole in China, it has been doing well in the international market. Consequently, intensive culture of tongue sole was developed and resulted in new fish disease in China. This paper reports isolation of *P. damselae* subsp. *piscicida* from diseased tongue sole in China for the first time.

2 MATERIALS AND METHODS

2.1 Experimental equipment and reagents

ABI 2700PCR machine (Advanced Biotechnologies Inc, Maryland, USA); TaKaRa agarose gel DNA purification kit (TaKaRa Biotechnology Co., Ltd, Dalian, P. R. China); PUCm-T vector (Shanghai Sangon); API 20E system (BioMrieux, France)

2.2 Isolation of pathogen

Diseased tongue sole, which exhibited haemorrhaging of the basal fin, yellowed kidney and ulceration over the

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body, was obtained from a fish farm in Qingdao, China. The fish were transported to the laboratory in seawater, and microbiological examination commenced with 2h of collection. Samples were taken aseptically from the hepatopancreas, blood, gall bladder, kidney, spleen and brain, and inoculated immediately onto marine agar 2216E (Difco) plates, which were incubated at 28℃ for 60h. After this, the dominant isolates were purified by streaking and re-streaking onto fresh media, with storage at −80℃ in sterile (100kPa 15min) 0.9% (W/V) saline supplemented with 15% (V/V) glycerol.

2.3 Fish pathogenicity

Overnight cultures on LB supplemented with 2% (W/V) NaCl were used to prepare suspensions in 0.85% (W/V) saline, which were counted by use of a haemocytometer slide (Improved Neubauer type) at a magnification of ×400. Also, the viable cell concentration was established by preparing serial 10-fold dilutions of the bacterial suspension in saline, and spreading 0.1mL volumes onto duplicate plates of LB supplemented with 2% (W/V) NaCl with incubation at 28℃ for 48h. Tongue sole from quarantined stocks recognized as disease-free[10] were used to assess pathogenicity. Groups of five fish (average weight = 95g) after inactivation (maintained at 9℃ ~ 11℃ for 5min) were infected by intraperitoneal (i.p.) injection with 200μL volumes of the bacterial suspensions. The fish were maintained in tanks containing aerated static seawater at 19℃ ~ 20℃, with a 50% water change daily. Controls were injected i.p. with 200μL volumes of saline. The infected fish were maintained for three weeks, and mortalities and the survives were examined microbiologically, as before. The lethal doses 50% were calculated using the Probit method[11]. Bacterial re-isolation was made on moribund tongue sole following the methods described in 2.2 and identification was made as previously described.

Zebrafish (Danio rerio) (average weight = 0.4 ~ 0.5g) was used as model for pathogenicity test following the methods described above. Bacterial re-isolation and identification was also made.

2.4 Characterization of the bacteria

A range of conventional phenotypic characters were studied[12]. In addition, the API 20E system (BioMrieux, France) was employed following the methods described in the product manual and incubated at 28℃ for 20h. The oxidase reactions were performed separately from the API 20E gallery.

2.5 Observation the bacteria with transmission electron microscopy

Bacterium was inoculated on LB (2% NaCl) at 28℃. The bacterium was harvested after 18h to 2mL Phosphate Buffered Saline (PBS) and centrifuged at 3000r/min for 2min, and the cells were resuspended in PBS and centrifuged at 3000r/min for 2min. The bacterium was then fixed with 2.5% glutaraldehyde. The bacterial solution was dripped onto a copper net and a few minutes later, stained for one to two minutes by 3% phosphotungstic acid then viewed through a transmission electron microscopy.

2.6 Antibiotic sensitivity

Antibiotic sensitivity was determined by using antibiotic discs impregnated with penicillin (10μg), oxacillin (1μg), ampicillin (10μg), neomycin (30μg), cefalexin (30μg), cefazolin sodium (30μg), cepahzolin (30μg), cifuroxime (30μg), amikacin (30μg), gentamycin (10μg), kanamycin (30μg), vancomycin (30μg), polymyxin B (300μg), midecamycin (30μg), ceftazidime (30μg), ceftriaxone sodium (30μg), piperacillinum (100μg), carbenicillin (100μg), tetracycline (30μg). The disc diffusion assay was carried out as follows: bacteria were harvested from 48h growth on LB agar (2% NaCl) (W/V) and suspended in 0.85% (W/V) saline corresponding to 10^8 cfu/mL. 0.1mL volumes of suspensions were streaked onto plates of LB supplemented with 2% (W/V) NaCl, and the discs were placed on the plates. After 48 h of incubation at 28℃, the diameters of the inhibition zones were measured. The numerical values of the zone sizes used as breakpoints given for each antimicrobial agent are pointed out in product manual. Based on diameters of inhibition zones, the antibiotic sensitivity of the isolate was determined.

2.7 16S rDNA PCR, sequencing and phylogenic analysis

The 16S rRNA gene of the isolate was amplified by PCR using the bacterial universal primers B8F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and B1510: 5′-GGTTACCTTGTTACGACTT-3′. The DNA template used for PCR was prepared as follows: a volume (1.5mL) of overnight bacterial culture was harvested by centrifugation at 5000r/min for 5min at 4℃, washed in 0.9% (W/V) saline, re-centrifuged, and the cell pellet was resuspended in 100μL of distilled water. The mixture was boiled for 10min, and centrifuged at 1200r/min for 10min to sediment the cell

debris. Then, the DNA-containing supernatants were transferred to fresh Eppendorf tubes.

The 16S rDNA fragment obtained from the PCR was purified using the TaKaRa agarose gel DNA purification kit (TaKaRa Biotechnology Co., Ltd, Dalian, P. R. China), and subsequently cloned into the PUCm-T vector (Shanghai Sangon). The recombinant plasmid containing the insert of expected size was sequenced by Shanghai Sangon. The 16S rDNA sequence was aligned and compared with available sequences in the NCBI GenBank using BLAST. MegAlign expert sequence analysis software from DNASTAR Inc. was used to construct phylograms. The 16S rDNA sequence of strain WY06 was submitted to GenBank with nucleotide accession number EF159147. Strains from different species of *Photobacterium*, *Vibrio hispanicus* and *Grimontia hollisae* were chosen to construct the phylogenetic tree based on 16S rDNA sequences. 16S rDNA sequences of these strains were obtained from GenBank.

3 RESULTS

3.1 Isolation and characterisation of the bacterial pathogen

Virtually pure dense cultures were obtained from gall bladder, blood, brain and hepatopancreas of diseased tongue sole on 2216E agar plates within 60 h of incubation. The isolate from gall bladder was designated WY06 and selected for subsequent study.

WY06 was a Gram-negative, rod shaped organism (Fig. 1). Growth did not occur on TCBS. On marine agar, the isolate formed smooth, circular, viscous, convex and raised translucent pale yellow colonies. WY06 had a relatively narrow range temperature and salinity tolerance, i.e. it did not grow at 4℃ and 37℃ or in 6% (W/V) NaCl. The data of A_{600} demonstrated that the optimal temperature and salinity for growth of WY06 were 20℃ and 2% (W/V) NaCl, respectively.

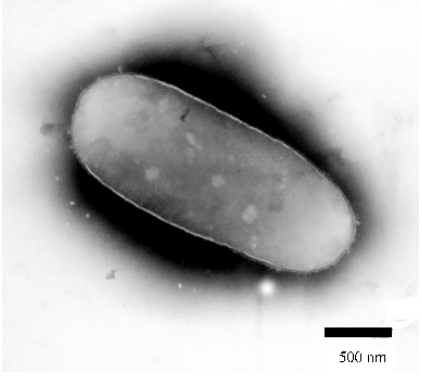

Fig. 1 Electron micrograph of WY06 after negative stain. WY06 appeared as bipolar rod shaped organism.

The organism gave positive reactions for arginine dihydrolase, Voges-Proskauer reaction, oxidase and fermentation of glucose, but was negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, H2S, tryptophan deaminase, urease, gelatinase, indole, citrate utilisation, and acid production from amygdalin, arabinose, inositol, sorbitol, rhamnose, melibiose, sucrose and mannitol, exhibited an API 20E index profile of 2005004 (Table 1). Table 1 also shows the physiological and biochemical characteristics of WY06 in comparison with 13 P. damselae subsp. piscicida strains from Japan, USA and Europe (included ATCC 17911 and ATCC 29690) as studied by Magariños *et al*[13].

Table 1　Characteristics exhibited by WY06 in comparison with *P. damselae* subsp. *piscicida*[13]

Phenotypic trait	*P. damselae* subsp. *piscicida* (Magariños *et al.*, 1992)	WY06
Gram stain	−	−
Motility	−	−
Oxidase	+	+
Voges-Proskauer reaction	+	+
Indole production	−	−
Citrate utilization	−	−
H_2S production	−	−
Glucose	+	+
Arginine dihydrolase	+	+
Lysine decarboxylase	−	−
Ornithine decarboxylase	−	−
Tryptophan deaminase	−	−
β-Galactosidase (*o*-nitrophenyl-β-D-galactopyranoside)	−	−
Urease	−	−
Gelatinase	−	−
Sucrose	−	−
Rhamnose	−	−
Arabinose	−	−
Amygdalin	−	−
Melibiose	−	−
Mannitol	−	−
Inositol	−	−
Sorbitol	−	−

+, positive reaction; −, negative reaction. WY06 exhibited an API 20E index profile of 2005004 which was identical with the profile exhibited by 13 Japanese, American, European strains in the study of Magariños *et al*[13].

3.2 Pathogenicity

WY06 was virulent for tongue sole fish with mortalities occurring between 3 and 19 d after i.p. injection. Most infected fish revealed haemorrhaging of the basal fin, ulceration of the body surface and sometimes anal prolapse. All fish was killed when 200μL volumes of WY06 containing 5.0×10^7 cells/mL were administered. In comparison, the 10-, 10^2-, 10^3- and 10^4-fold-diluted suspension of WY06 killed 100%, 40%, 0% and 0% of the tongue sole, respectively (Table 2). Thus, the LD_{50} was established as 5.5×10^3 cfu/g of fish (5.2×10^5 cfu/fish). In addition, WY06 was reisolated as pure cultures from internal organs.

Table 2 Pathogenicity to tongue sole

Concentration of bacteria/(cfu/mL)	Dosage/μL	Total mortality (n = 5)	Time to death/d
5.0×10^7	200	5	3 ~ 4
5.0×10^6	200	5	6 ~ 16
5.0×10^5	200	2	11 ~ 19
5.0×10^4	200	0	21
5.0×10^3	200	0	21
Control	200	0	21

Groups of 5 tongue sole (average weight = 95g) were infected by i.p. injection with 200μL volumes of WY06 containing 5.0×10^3 cells/mL ~ 5.0×10^7 cells/mL. Controls were injected with 200μL volumes of saline.

WY06 was virulent for zebra fish and the LD_{50} was established as 1.9×10^3 cfu/g of fish (8.9×10^2 cfu/fish) (Table 3). Although gross external signs of disease were not observed in zebrafish, they all presented haemorrhages in the intestinal tract. WY06 was also reisolated as pure cultures from internal organs.

3.3 Antibiotic sensitivity

WY06 was sensitive to cifuroxime (30μg) and ceftriaxone sodium (30μg). It was moderately susceptible to neomycin (30μg), gentamicin (10μg) and vancomycin (30μg) and resistant to the other antibiotics.

Table 3 Pathogenicity to zebrafish

Concentration of bacteria/(cfu/mL)	Dosage/μL	Total mortality (n = 5)	Time to death/h
2.5×10^6	20	5	12 ~ 60
2.5×10^5	20	4	24 ~ 98
2.5×10^4	20	2	108 ~ 132
2.5×10^3	20	0	336
Control	20	0	336

Groups of 5 zebrafish (average weight = 0.4g ~ 0.5g) were infected by i.p. injection with 20μL volumes of WY06 containing 2.5×10^3 cells/mL ~ 2.5×10^6 cells/mL. Controls were injected with 20μL volumes of saline.

3.4 Analysis of 16S rRNA gene

The comparison with known 16S rDNA sequences in the GenBank using the BLAST program showed that the sequence of WY06 was most similar (99%) to those of members of *P. damselae* subsp. *piscicida*. In the phylogenetic tree, WY06 branched together with *P. damselae* subsp. *piscicida* and formed a subgroup with the taxon which was clearly phylogenetically distinct from the other *Photobacterium* species. However, it is important to note that *Grimontia hollisae* grouped into *Photobacterium* species (Fig. 2).

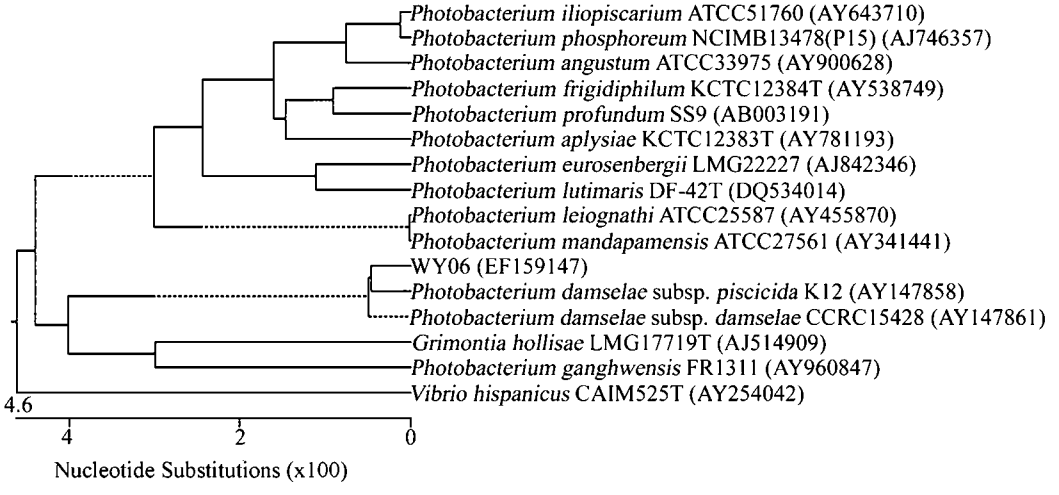

Fig.2 Phylogenetic tree based on 16S rDNA sequences. The phylogenetic tree was constructed by the MegAlign expert sequence analysis software from DNASTAR based on the 16S rDNA sequence. WY06 branched together with *P. damselae* subsp. *piscicida* strain K12. The outgroup is *V. hispanicus*.

4 DISCUSSION

Isolate WY06 from diseased tongue sole has the properties of *P. damselae* subsp. *piscicida*, and exhibited an API 20E index profile of 2005004 (Table 1) which was identical with the profile exhibited by all Japanese, American, European strains in the study of Magariños *et al*[13]. Kent[14] suggested that, although the pathogen is not included in the API-20E code index, the system is valuable for a rapid presumptive identification of the bacterium, because all strains have a similar pattern (2005004), neither false-positive nor false-negative reactions being detected[15]. However, Thyssen *et al*.[16] studied 113 *P. damselae* subsp. *piscicida* strains, with only 76% (86 out of 113) exhibiting this profile. Based on these findings, they concluded that the API 20E test system is not always a useful tool for identification of *P. damselae* subsp. *piscicida*. Confirmation of its identity was provided by its strict salt requirement, and narrow range of growth temperatures.

The high levels of 16S rDNA sequence similarity between WY06 and established *P. damselae* subsp. *piscicida* demonstrated that WY06 should be a member of the taxon. *Grimontia hollisae* was not an outgroup, but clustered with *P. damselae* and *P. ganghwensis*. The result was supported by studies of Gauthier *et al*.[17] who recommended that reassignment of *V. hollisae* (*Grimontia hollisae*) and *V. costicola* to the genus *Photobacterium* should be considered on the basis of the results of 16S rRNA gene sequence comparisons and the ability of the organisms to accumulate polyhydroxybutyrate, which is characteristic of the species belonging to the genus *Photobacterium*. However, the taxonomic status of *Grimontia hollisae* is still required further study which could be based on other genes.

The zebrafish has become recognized as a valuable model for the study of development, genetics and toxicology. Recently, the zebrafish has been recognized as a useful model for infectious disease and immunity[18]. In this study, the LD_{50} of WY06 for zebrafish and tongue sole were approximately identical. This demonstrated that zebrafish was used successfully for determining pathogenicity.

Previous study[17] demonstrated that strains of *P. damselae* subsp. *piscicida* were sensitive to tetracycline. However, WY06 was resistant to tetracycline. The difference of the antibiotic sensitivity might reflect the fact that this drug was commonly used to treat bacterial diseases in China. In addition, Thyssen and Ollevier[19] reported that there was a correlation between antibiotic resistance and geographical origin of the isolates. While all American and European isolates were sensitive to kanamycin, 49% of the Japanese isolates were resistant to it. WY06 was also resistant to kanamycin.

P. damselae subsp. *piscicida* is a highly pathogenic bacterium that does not seem to have host specificity. *P. damselae* subsp. *piscicida* was isolated from wild populations of white perch (*Morone americanus*) and striped bass (*Morone saxatilis*) for the first time. A few years later, it affected yellowtail (*Seriola quinqueradiata*) and ayu (*Plecoglosus altivelis*) cultures in Japan[20,21]. In 1990, an epizootic caused by the bacterium *P. damselae* subsp. *piscicida* occurred in gilthead sea bream (*Sparus aurata*) in northwestern Spain[3,4]. In the same period, outbreaks of pasteurellosis also occurred in France[8], Italy[9], affecting different marine fish, mainly gilthead sea bream and sea bass (*Dicentrarchus labrax*). However, at present time *P. damselae* subsp. *piscicida* was isolated from diseased tongue sole. Therefore, pasteurellosis can be a risk even for marine fish species in which the disease has not been yet described.

In conclusion, *P. damselae* subsp. *piscicida* has been clearly demonstrated to be pathogenic to tongue sole. Moreover, this is the first time that this pathogen has been identified in China.

REFERENCES

[1] Snieszko SF, Bullock GL, Hollis E, *et al*. *Pasteurella* sp. from an epizootic of white perch (*Roccus americanus*) in Chesapeake Bay tidewater areas. *J Bacteriol*, 1964, **88**: 1814 – 1815.

[2] Kusuda R, Miura W. Characteristics of a *Pasteurella* sp. pathogenic for pond cultured ayu. *Fish Pathol*, 1972, **7**: 51 – 57.

[3] Toranzo AE, Barreiro S, Casal JF, *et al*. Pasteurellosis in cultured gilthead seabream, *Sparus aurata*: first report in Spain. *Aquaculture*, 1991a, **99**: 1 – 15.

[4] Toranzo AE, Santos Y, Núñez S, *et al*. Biochemical and serological characteristics, drug resistance, and plasmid profiles of Spanish isolates of *Aeromonas salmonicida*. *Fish Pathol*, 1991b, **26**: 55 – 60.

[5] Magariños B, Toranzo AE, Romalde JL. Phenotypic and pathobiological characteristics of *Pasteurella piscicida*. *Annu. Rev Fish Dis*, 1996, **6**: 41 – 64.

[6] Magariños B, Romalde JL, López-Romalde S, *et al*. Pathobiological characterization of *Photobacterium damselae* subsp. *piscicida* strains isolated from cultured sole (*Solea senegalensis*). *Bull. Eur. Assoc. Fish Pathol*, 2003, **23**: 183 – 190.

[7] Zorrilla I, Balebona MC, Moriñigo MA, *et al*. Isolation and characterization of the causative agent of pasteurellosis, *Photobacterium damsela* ssp. *piscicida*, from sole *Solea senegalensis* (Kaup). *J. Fish Dis*, 1999, **22**: 167 – 171.

[8] Baudin-Laurencin F, Pepin JF, Raymond JC. First observation of an epizootic of pasteurellosis in farmed and wild fish of the French Mediterranean coasts. Abstr. 5th Int. Conf. Eur. Assoc. Fish. Pathol, 1991, Budapest, Hungary, abstr., p. 17.

[9] Ceschia G, Quaglio F, Giorgetti G, *et al*. Serious outbreak of pasteurellosis (*Pasteurella piscicida*) in euryhaline species along the Italian coasts. Abstr. 5th Int. Conf. Eur. Assoc. Fish. Pathol, 1991, Budapest, Hungary, abstr., p. 26.

[10] Austin B, Austin DA. Methods for the Microbiological Examination of Fish and Shellfish. Ellis Horwood, Chichester, 1989.

[11] Wardlaw, AC. Practical statistics for experimental biologists. John Wiley and Sons, Chichester, United Kingdom, 1985.

[12] Austin B, Austin DA. Bacterial Fish Pathogens. Diseases of Farmed and Wild Fish, 3rd (revised). Godalming: Springer-Praxis, 1999.

[13] Magariños B, Romalde JL, Bandin I, *et al*. Phenotypic, antigenic, and molecular characterization of *Pasteurella piscicida* strains isolated from fish. *Applied and Environmental Microbiology*, 1992, **58**: 3316 – 3322.

[14] Kent ML. Characteristics and identification of *Pasteurella* and *Vibrio* pathogenic to fishes using API-20E (Analytab Products) multitube test trips. *Can J Fish Aquat Sci*, 1982, **39**: 1725 – 1729.

[15] Santos Y, Romalde JL, Bandín I, *et al*. Usefulness of the API-20E system for the identification of bacterial fish pathogens. *Aquaculture*, 1993, **116**: 111 – 120.

[16] Thyssen A, Grisez L, van Houdt R, *et al*. Phenotypic characterization of the marine pathogen *Photobacterium damselae* subsp. *piscicida*. *International Journal of Systematic Bacteriology*, 1998, **48**: 1145 – 1151.

[17] Gauthier G, Lafay B, Ruimy R, *et al*. Small-subunit rRNA sequences and whole DNA relatedness concur for the reassignment of *Pasteurella piscicida* (Snieszko *et al*.) Janssen and Surgalla to the genus *Photobacterium* as *Photobacterium damsela* subsp. *piscicida* comb. nov. *Int. J. Syst. Bacteriol*, 1995, **45**: 139 – 144.

[18] Phelan PE, Pressley ME, Witten PE, *et al*. Characterization of snakehead rhabdovirus infection in Zebrafish (*Danio rerio*). *Journal of Virology*, 2005, **79**: 1842 – 1852.

[19] Thyssen A, Ollevier F. In vitro antimicrobial susceptibility of *Photobacterium damselae* subsp. *piscicida* to 15 different antimicrobial agents. *Aquaculture*, 2001, **200**: 259 – 269.

[20] Kimura T, Kitao T. On the causative agent of tuberculosis of yellowtail. *Fish Pathol*, 1971, **6**: 8 – 14.

[21] Kubota S, Kimura M, Egusa S. Studies of a bacterial tuberculosis of the yellowtail. I. Symptomatology and histopathology. *Fish Pathol*, 1970, **4**: 11 – 18.

半滑舌鳎病原菌(发光杆菌杀鱼亚种)的分离与鉴定

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摘 要:2006 年夏,山东青岛某渔场养殖半滑舌鳎(*Cynoglossus semilaevis* Gunther)大量死亡。症状主要表现为体表溃烂,鳍基部出血等,解剖可见胆囊发黑,肾脏发黄。从患病半滑舌鳎胆囊分离出优势菌并命名为 WY06。人工感染试验证实 WY06 对半滑舌鳎及模式动物斑马鱼都具有很强的致病性,其半数致死量分别为 5.5×10^3 cfu/克鱼(5.2×10^5 cfu/条鱼)和 1.9×10^3 cfu/克鱼(8.9×10^2 cfu/条鱼)。该病原菌革兰氏染色阴性,菌体呈杆状。综合该菌在形态、生理生化特征及 16S rDNA 同源性等方面的结果,确认 WY06 为美人鱼发光杆菌杀鱼亚种(*Photobacterium damselae* subsp. *piscicida*)。该菌对头孢呋肟、菌必治等抗生素敏感。美人鱼发光杆菌杀鱼亚种在美国、日本、欧洲的海水养殖中为常见的病原菌,但作为鱼类病原菌在中国属首次报道。

关键词:美人鱼发光杆菌杀鱼亚种;*Photobacterium damselae* subsp. *piscicida*;巴斯德氏菌病;半滑舌鳎;致病性;16S rDNA

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